THE LIFE HISTORY OF THE AUSTRALIAN OYSTER (*OSTREA COMMERCIALIS**).

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(Plates x–xxvii; two Text-figures.)

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Introduction.

The history of the oyster in every country of its occurrence shows that at some time or other the supply has fallen short of the requirements demanded by commerce, and always scientific aid has been sought in an endeavour to account for the depletion, and if possible to provide means for ensuring that a similar shortage might be avoided in the future. Naturally, the propagation of the oyster, involving the study of its life history from the fertilization of the egg till the larval oyster attaches as a spat, when it comes under the control of the cultivator, has on this account received extensive study. Embryological oyster research had its origin in France, where very serious shortages occurred about the middle of last century, at a time when science itself had made sufficient progress to enable the problems attendant on such a subject to be investigated with some degree of accuracy. Ever since that time the life history of the oyster has been the object of specialized study by a large number of zoologists, and a considerable amount of literature on the subject has accumulated. Mention will be made here of a few only of the more important discoveries.

In 1852 the most notable advance up to that time was made by Davaine, who found that the oyster of northern Europe (*Ostrea edulis*) was hermaphrodite, being alternately male and female. In 1858 Coste made the first attempt to develop oysters by artificial means, but his efforts proved to be entirely negative, for the embryonic and early larval stages are passed in the mantle cavity of the parent, and the artificial fertilization of the egg is rarely, if ever, successful. It was demonstrated, however, by Bouchon-Brandely (1882) that the small Portuguese oyster, *O.* (*Gryphaea*) *angulata*, was dioecious, and that fertilization readily occurred when the eggs from the female and the sperms from the male were artificially extracted from the parents and intermingled in sea water.

In addition to these workers, there have been many others who have assisted in the elucidation of the problems connected with the embryology of the European species. Amongst these may be mentioned Lacaze-Duthiers (1854), Van Beneden (1855), Möbius (1871, 1877), Horst (1882, 1884), Huxley (1883) and Hoek (1883).

The period 1883 to 1921 was one of little activity, but in the latter year Orton began a series of researches which have elucidated many of the problems unsolved by earlier workers. Notable, too, is the work of Spärck (1925).

It was not till 1879 that American scientists began to display an interest in the life history of the American species (*O. virginica*). In that year Brooks began a detailed study of the embryology of the oyster. It was he who first showed that this species was dioecious, the eggs and sperms being ejected direct into the water, where fertilization takes place, and the free-swimming stages of development are undergone before the larva attaches itself and becomes a spat.

* This is the common commercial oyster of Australia for which the specific name *O. cucullata* has usually been adopted (see page 278).

Brooks has been followed by a long line of distinguished investigators, of whom mention may be made of Ryder (1881), Rice (1883), Winslow (1884), Jackson (1888), J. Nelson (1888–1917), and more recently by T. C. Nelson (1916–1932), Churchill (1919), Wells (1920), Prytherch (1923), Galtsoff (1930); and in Canada, by Stafford (1905–1917).

Stafford's researches are notable for their comprehensiveness, and his discovery (1907) that the free-swimming period extends from three to four weeks in Canadian waters was not suspected by most earlier workers. But previous investigators had invariably tried to develop the young oysters through the free-swimming period from artificially fertilized eggs, and endeavoured to deduce the complete length of larval life from the time taken to reach the stage when their cultivated larvae died. Stafford, however, by means of a plankton net towed over the beds daily, was able to follow the development of the larvae under natural conditions and watch the daily growth-rate till they settled down as spat. Having obtained the larvae in every stage of growth, Stafford was afforded an opportunity of studying their structure and movements at ages and sizes previously unrecognized. Amongst other discoveries he found (1905) that in its later larval life the oyster develops a foot by means of which it can crawl about quite vigorously; he also identified a byssus gland at the base of the foot, and reasoning by analogy with the function of such glands in other Lamellibranchs, he came to the conclusion that in the oyster it produced the material which cemented the shell to an object when the larva attached itself to become a spat. This was shown by T. C. Nelson (1924) to be a correct deduction; he actually saw larvae settle down on glass with the left shell valve held in contact with the substratum by the foot while the secretion from the byssus gland was distributed by means of the protruded mantle.

J. Nelson, in New Jersey, studied the daily growth-rate of normally occurring larvae by filtering the water from the oyster beds. He found (1908) that the free-swimming stage varied from two to three weeks according to the temperature. Later (1915) he determined that at ordinary summer temperatures an interval of from 13 to 16 days elapsed between the fertilization of the egg and the subsequent attachment as spat. This has been confirmed many times by more recent workers.

It has been the aim of practically every American investigator to raise spat from artificially fertilized eggs, and several had announced that this had been accomplished. The size to which they had developed their larvæ showed, as Stafford points out, that in no instance had development proceeded far enough to enable fixation to take place. The great trouble experienced by all workers was filtration. A means had yet to be devised whereby a continuous flow of water would be permitted without losing the microscopic larvae. It remained for Wells (1920) to accomplish this feat. He was able, by concentrating the larvae at intervals in a small amount of water, by means of a milk separator, and then transferring them to fresh sea water, to carry large numbers of larvae through to the setting stage. In 1920 a thousand spat were obtained; in 1923, by improvements in the technique, ten thousand spat were produced; in 1925 a total of a hundred thousand spat was reached; and during 1926 a million larvae successfully set as spat.

Prytherch also, in 1923, succeeded in raising spat by artificial propagation. He found that the problem of filtration was solved to a great extent by the use of a new material known as "filtros", a white, rigid, porous, artificial stone, composed

essentially of silica, and similar to a sand filter in block form. The various grades of porosity in which this material is made rendered it possible to retain organisms of any definite size and still allow a good flow of water. For the retention of the embryos a combination filter of loose, fine sand and filtros was used until the embryo oysters had reached the shell stage; from that time on the larvae were held in by the filtros blocks of various grades, which were changed to suit the growth and size of the forms, and when the larvae were ten days old, or older, they were held in by means of fine monel metal screens, which permitted an unusually good flow of water. By this means large numbers of spat were developed in from fifteen to twenty days from oysters which spawned naturally on trays suspended just beneath the surface of the water.

The increasing success achieved by these experiments leads to the expectation that eventually the artificial propagation of oysters may be carried out on a large scale commercially, thereby eliminating the costly uncertainty which up to the present has always attended the spatting season. Moreover, it is hoped that, by selective breeding, the quality, shape and size of the oysters may be considerably improved.

Of recent years considerable attention has been given to the oyster by Japanese workers and much light has been thrown on the embryology of several species occurring in Japanese waters, particularly on that of the common commercial oyster, *O. gigas*. Prominent amongst these workers are Hori and Kusakabe (1926), Amemiya (1928), and Seno (1929).

The Indian oyster (*O. cucullata*) received practically no attention until 1931, when Awati and Rai published much valuable information on its structure and breeding habits.

The world's oysters may be divided into two main types: 1, those which retain the embryos and larvae for a considerable time in the mantle cavity of the parent, later ejecting the shelled larvae into the water where their larval development is completed (larviparous type); and 2, those which spawn direct into the water, where the fertilization of the egg is accomplished and where the whole of the larval life is spent till attachment takes place (oviparous type). Examples of larviparous oysters are *O. edulis* of Europe; *O. lurida* of the Pacific coast of America; *O. denselamellosa* of Japan; *O. angasi* of southern Australia and Tasmania; and *O. lutaria* of the South Island of New Zealand. Examples of oviparous oysters are *O. angulata* of Portugal (which has largely replaced the indigenous *O. edulis* on the beds of France); *O. virginica* of the Atlantic coast of America; *O. gigas* of Japan; *O. cucullata* of India; and *O. commercialis* of Australia.

As long ago as 1852 it was shown that the larviparous European oyster (*O. edulis*) was hermaphrodite, inasmuch as its sex was not constant, but definitely changed at least from female to male. It has been shown by more recent workers (Orton, Spärck) that during the course of its life, *O. edulis* undergoes a series of sex changes, beginning as a male and alternating as male and female throughout life, and described by Spärck as an alternating protandic hermaphrodite.

In the case of the oviparous type, Brooks (1879) found that the oviparous *O. virginica* was unisexual, each oyster remaining a male or female throughout life. And since that date all oviparous oysters have been regarded as unisexual. Kellogg, however, in 1890, recorded an individual of *O. virginica* whose gonad contained both ova and sperms, and Amemiya in 1925 described three hermaphro-

dite individuals of the oviparous Portuguese oyster (*O. angulata*). In 1928, the writer recorded nine hermaphrodite individuals in *O. commercialis* (*cucullata*), and was able to show for the first time that a sex-change occurs in this oviparous species. Later in the same year Amemiya recorded cases of abnormal hermaphroditism in the oviparous Japanese oyster (*O. gigas*), and the following year (1929) showed that a sex-change occurs in this species also. Early in 1931 Awati and Rai recorded a sex-change amongst numbers of the Indian oviparous species (*O. cucullata*).

Thus, during three years, three species of oviparous oysters, long regarded as unisexual, have been shown to have a sex-change. But what of the other oviparous species? The recorded occurrence of a hermaphrodite individual of *O. virginica*, and of three individuals of *O. angulata*, raises the question whether these species may not also change their sex. Further research may yet disclose that no species of the genus *Ostrea* is unisexual.

It has been customary to regard the larviparous oysters as monoecious, and the oviparous as dioecious, but in the light of recent research these terms are no longer tenable, and it will save confusion if the terms "monoecious" and "dioecious" are avoided.

In Australia the study of the life history of the oyster has received scant attention. Tenison-Woods (1883) stated that the sexes are divided and the eggs are probably discharged into the water where they may easily meet with male cells. By mixing "the male and female fluid" it was found that fertilization of the ova readily occurred.

Later, in 1888, Tenison-Woods appears to have altered his views concerning the discharge of the sexual products into the water, and stated that "young oysters are reared in the gill-chambers of the mother, in the case of the Australian oyster, *O. mordax*". It seems probable that Tenison-Woods was confused in his species, for the taxonomy of the New South Wales species was at that time very unstable. In the first instance he was apparently dealing with the oviparous *O. commercialis*, and in the second with the larviparous *O. angasi*, both of which were then plentiful in the neighbourhood of Sydney.

Tenison-Woods believed that when expelled from the parent, the young were sufficiently developed to be able to affix themselves as spat. He also stated that the veliger stage possessed a ring of cilia and in the centre a long flagellum. He was obviously unable to distinguish oyster larvae from those of other bivalves, for at no period of its development does the oyster larva possess a flagellum, though one is present in mussel larvae and forms a provisional byssus.

Altogether, Tenison-Woods' observations were quite confusing and threw little real light on the subject.

The work of Saville-Kent (1890), although it erred in a number of particulars, contained much of value. He stated that in the case of *O. glomerata* (*commercialis*) fertilization takes place in the water, "the young embryos . . . being thrown upon their own resources from the earliest period of their existence", while the embryos of *O. angasi* are retained within the mantle cavity of the parent, as are also those of the oyster of the south of New Zealand, which he regarded as a local variety of *O. angasi*. Saville-Kent described the embryonic development of *O. commercialis* as far as the complete envelopment of the embryo by the shells, but erroneously assumed and actually illustrated their attachment at that stage as spat, stating that the length of larval life occupied, under favourable conditions, four days. His figure 21 shows the "earliest observed

attached condition of the oyster embryos or "spat", attained to within the fourth or fifth days succeeding the primary fertilization of the ovum; magnified about 50 diameters". These so-called "spat" are shown 4·5 mm. wide; they represent, therefore, individuals 0·09 mm. wide, or between one-third and one-fourth the size of fully developed larvae or the earliest attached spat. Actually, they are young shelled larvae of the straight-hinge stage, quite incapable of attachment.

Such, then, was the state of our knowledge of the life history of the Australian oyster till the writer published a popular description in the *Australian Museum Magazine* in 1925.

The present researches have been carried on intermittently since 1924. It is hoped that opportunity will allow of their continuance, for much remains to be done, and a particularly interesting problem awaits solution in the working out of details of the sex-change. It is clearly shown that a change from male to female occurs in the majority of oysters, but it is not known whether all of the oysters change their sex, or whether any of them change from female to male. I hope to have the opportunity of studying that problem in the near future.

The oyster with which this paper deals is the Australian edible oyster of commerce. It appears to be confined to the eastern Australian coast, its range extending from the far North Queensland coast to as far south as Wingan Inlet in Victoria. In Queensland, the principal source of supply is in Moreton Bay, where large quantities are grown and matured for market. Prolific crops occur in Port Curtis and Sandy Bay, but they rarely thrive there owing mainly to a too high salinity. Considerable quantities are transferred to Moreton Bay, however, where growth becomes much more rapid.

This oyster thrives best in those estuaries and rivers flowing to the east coast of New South Wales which are fed by an abundance of fresh water from their sources. The optimum water density lies between 1·015 and 1·020.

From New South Wales, where its cultivation is an important industry, considerable quantities are exported to Victoria, South Australia and Western Australia, its splendid keeping qualities allowing long distances to be travelled without deterioration.

In Victoria, it occurs only in one or two rivers, principally Wingan Inlet, on the extreme east, but there is no industry engaged in its cultivation in that State.

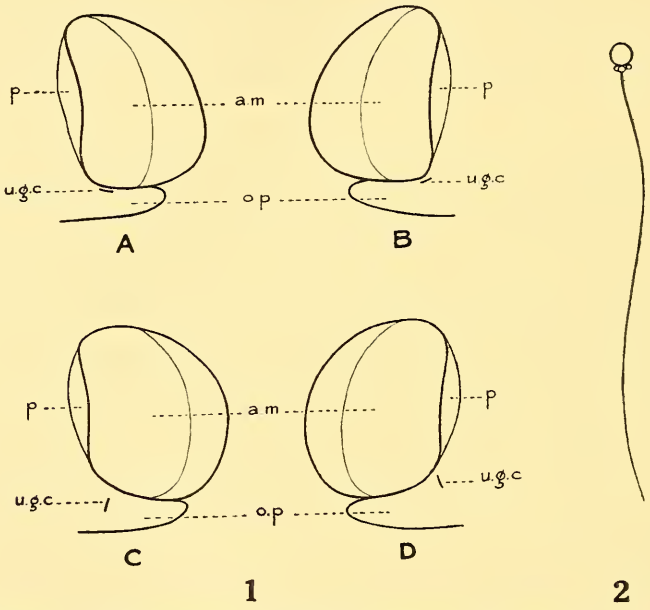
The only other Australian oyster which enters into commerce is the larviparous *O. angasi*, which resembles very closely the European *O. edulis* and the Japanese *O. denselamellosa*. Small quantities only are marketed from the waters of Victoria, South Australia and Tasmania.

The External Appearance of the Gonad.

Externally the gonad, when well developed, appears as an enormous cream-coloured organ extending from the anterior extremity of the animal, in close proximity to the hinge, back as far as the pericardium. If the mantle is cut away below the adductor muscle the gonad will be seen to continue along the oral process to about two-thirds of its length. It is on the lateral walls of the oral process that the gonoducts reach the exterior (Pl. x, fig. 1); they open into slit-like depressions, one on each side. The same depressions serve as outlets for the kidneys (organs of Bojanus), and they may therefore be designated urino-genital clefts.

In *O. commercialis* the cleft of the left side is situated a little behind the anterior wall of the adductor muscle (Text-fig. 1, *u.g.c.*); it lies in the middle line, or a little above the middle line, of the oral process and crosses it almost transversely, the dorsal edge being a little posterior to the ventral. On the right side it is situated a little farther forward, and lies immediately beneath the pericardium and in close proximity to the adductor muscle; on this side, also, it crosses the long axis of the oral process transversely.

The disposition of the urino-genital cleft in this oyster varies somewhat from that of *O. edulis*. An examination of several individuals of this species, sent to me by Professor J. H. Orton, of the Liverpool University, shows that the urino-genital cleft opens longitudinally on the oral process on both sides in close apposition with the adductor muscle (Text-fig. 1, A, B, *u.g.c.*).



Text-fig. 1.—The situations of the urino-genital clefts of *O. edulis* and *O. commercialis* compared. A. *O. edulis*, left side; B. *O. edulis*, right side; C. *O. commercialis*, left side; D. *O. commercialis*, right side. *a.m.*, adductor muscle; *o.p.*, oral process; *p.*, pericardium; *u.g.c.*, urino-genital cleft.

Text-fig. 2.—Spermatozoon of *Ostrea commercialis*. × 1,500.

The gonoduct, as it extends forward on each side, immediately begins to give off branches which coalesce and extend in all directions just beneath the surface. In most ripe gonads these can be plainly seen; in some (Pl. x, fig. 2) they are very prominent, but occasionally an individual will be found with a well developed gonad in which the ducts beneath the surface are practically indistinguishable (Pl. x, fig. 3). Such a uniform gonad is frequently indicative of maleness, and one with prominent ducts will usually be found to be an ovary, but the sex cannot be judged with certainty by this means, for I have occasionally seen males with extremely prominent ducts.

The gonads of the oyster are usually described as consisting of two in number, one on each side, but so extensively do the ducts from one side coalesce with

those from the other side along the dorsal and anterior surfaces, and also the follicles internally, that it is impossible to regard them as two entirely separate organs. The disposition of the ducts shows that the bulk of the sexual products produced on the left side find their way to the urino-genital cleft on that side, and similarly with the right side, but it would be quite impossible to say through which opening the genital products which are matured along the dorsal and anterior regions of the gonad would emerge.

When the oyster begins to spawn the anterior region of the gonad is usually the first to be drained (Pl. xi, fig. 4). After a partial spawning (Pl. xi, fig. 5) the gonad appears as a very irregular mass; areas on each side may be completely depleted, and others may retain the sexual products intact. If the spawning is complete, the organs which were formerly obscured by the gonad, such as the digestive diverticula and the intestine, become clearly distinguished through the thin layer of vesicular connective tissue which covers them.

The Relationship of the Gonad with other Internal Organs.

The gonad is frequently spoken of as completely surrounding the digestive organs; careful dissection, however, shows that this is not strictly correct, for in the region where the digestive diverticula abut on the labial palps and gills the gonad terminates on each side at the point of attachment of the mantles. The relationship of the gonad to the organs which it partially encloses can be studied quite effectively by slicing off with a razor sections about an eighth of an inch thick, beginning at the anterior extremity and working backwards to the termination of the oral process (Pl. xii, fig. 6). Examined with the aid of a binocular microscope with a magnification of about 20 diameters, the disposition of the principal organs can be clearly distinguished.

In the region nearest the hinge the gonads of the right and left sides are confluent and appear as a homogeneous mass enclosed by a layer of vesicular connective tissue which shows as a whitish band beneath the external epithelium.

In the region of the mouth, the digestive diverticula make their appearance above the mouth, but are separated from it by a horizontal layer of the gonad. Toward the anterior end of the oesophagus the digestive diverticula completely surround it, and are themselves surrounded by the gonad above and on the sides; the gonad, however, does not extend ventrally into the area between the oesophagus and the labial palps. Towards the posterior extremity of the oesophagus, the digestive diverticula no longer completely surround it; they are absent from the dorso-lateral region on the left side. Here the gonad is separated from the oesophagus by vesicular connective tissue only. Ventrally, the gonad on each side terminates at the origin of the mantles, a large area of digestive diverticula separating the gonads of the right and left sides, and lying in intimate contact with the dorsal margins of the palps.

In a vertical plane above the anterior extremities of the gills, the digestive diverticula are confined to the right side and to the horizontal area above the gills. The gonad on the left side extends inwards almost to the oesophagus, from which it is separated by a layer of vesicular connective tissue; it forms a solid mass dorsally, and lies external to the digestive diverticula on the right side. On the left side, and to a less extent on the right side, the gonad now begins to invade the horizontal area above the gills.

In the region of the stomach the digestive diverticula occur in a dense mass on the right side and below the stomach; on the left side they occur in islands

bounded by vesicular connective tissue. Here the gonad still further penetrates the area above the gills on both right and left sides.

Towards the posterior region of the stomach, the digestive diverticula do not lie in such close apposition with the stomach wall, but are separated from it by a layer of vesicular connective tissue which shows as a prominent band; they enclose the stomach everywhere except dorsally. The gonad now almost completely surrounds the digestive diverticula; the inwardly extending areas above the gills, however, have not yet joined above the two inner hemibranchs.

Soon after the mid-gut leaves the stomach the digestive diverticula no longer appear on the right side of the gut; they and the mid-gut are completely surrounded by the gonad, the area above the gills now consisting of a continuous mass of gonadial tissue. Within a short distance the digestive diverticula terminate on the left side, and the gonad then forms a compact mass surrounding the mid-gut and the returning loop of the intestine, and separated from them by a relatively narrow area of vesicular connective tissue.

In the oral process, the mid-gut and returning intestine lie in the same horizontal plane and in close apposition on the right side. In *O. edulis* the mid-gut lies above the returning loop of the intestine. The gonad on the left side continues farther along the oral process than on the right side, and a little distance beyond the urino-genital cleft.

Such are the principal relationships of the gonad with the digestive organs. It is seen that it completely surrounds the digestive diverticula only in the region behind the stomach. It is traversed by numerous blood vessels, the dorsal vessel or anterior aorta showing prominently in all the sections cut anterior to the pericardium except those closest to the hinge, where it divides into three parts. Posteriorly, the anterior aorta lies in the centre of the mass of gonadial tissue situated above the mid-gut, and as it travels forward it works slightly to the left side, above the stomach and oesophagus. The posterior aorta becomes conspicuous in sections in front of the pericardium where it is seen to descend in close proximity to the mid-gut.

The loop of the intestine anterior to the stomach lies usually immediately above the digestive diverticula, though occasionally it may traverse their uppermost offshoots; it lies in the median vertical plane or slightly on the right side until it begins to descend, when it crosses over to the left side and runs backwards in close apposition with the ventro-lateral border of the digestive diverticula. It is embedded throughout its length in a layer of vesicular connective tissue. This disposition of the intestine differs from that of *O. edulis*; in that species the intestine, after leaving the oral process, crosses over to the left side and lies above and to the left of the stomach, the anterior loop descending vertically.

The gonad when fully developed consists of anastomosing follicles with a series of ducts on the surface, which serve to convey the ripe eggs or sperms to the urino-genital cleft. In the very young oyster, in which the sexual products are beginning to develop for the first time, the gonad consists solely of a series of branching ducts which run in an antero-posterior direction; they lie very close to the surface. The study of the disposition of these ducts can only be done by a series of microscopic sections, and the description which follows has been drawn from such a series in an oyster about six months old, which was just beginning to produce sex cells. The sections were cut transversely, beginning at the hinge end and terminating at the posterior edge of the mantle.

In sections of the anterior extremity of the body, the ducts form a complete circle and are separated from the surface epithelium by a comparatively thick layer of vesicular connective tissue, which, much condensed in proximity to the external walls of the ducts, forms a continuous and fairly thick band external to them. The area enclosed by the ducts is composed of vesicular connective tissue only. In the region anterior to the mouth, above the dome-shaped anterior extremities of the palps, the digestive diverticula make their appearance. The ducts of the gonad surround them above and on the sides, but they do not extend into the vesicular tissue which separates the digestive diverticula from the mantle cavity.

In the plane where the mouth gives place to the oesophagus to form a hollow tube compressed dorso-ventrally (Pl. xii, fig. 7), the digestive diverticula lie in a compact mass in the region above the oesophagus, which is separated from them by a thick layer of vesicular connective tissue. The ducts of the gonad do not penetrate this layer, but terminate on each side above the attachment of the right and left mantles, which in this region are confluent and form a sort of hood over the palps.

As the oesophagus proceeds backwards and upwards it shortly enters the region of digestive diverticula, which then come to surround it on all sides. As the ducts of the gonad extend backwards, they gradually approach closer to the external epithelium, until, about half-way along the oesophagus, they are separated from it by a very thin layer of vesicular connective tissue which in places scarcely exists at all; the ducts are now lenticular or elliptical in cross section, with the long axis in some cases considerably produced. Their size varies greatly.

In the plane above the posterior extremities of the palps, or the anterior extremities of the gills, which traverses the junction of the oesophagus and stomach, the relative positions of the various organs have undergone little alteration. The ducts of the gonad form a ring under the surface epithelium above and on the sides, and still terminate above the junction of the mantles or outer hemibranchs of the gills. A layer of vesicular tissue of irregular thickness separates them from the digestive diverticula, except on the left side, where in this region the digestive diverticula are absent. The ducts of the right and left sides are separated inferiorly by a thick layer of digestive diverticula which occupies the whole of the space between the stomach and the attachment of the gills.

In a vertical plane which traverses the anterior portion of the stomach, the left kidney makes its appearance as a follicle situated above the junction of the mantle with the body. It is shaped like an elongated duct of the gonad and lies external to the lowermost of those ducts, between it and the external epithelium. Proceeding backwards, other follicles of the left kidney soon appear and extend inwards along the horizontal layer of vesicular tissue which occupies the region between the digestive diverticula and the gills. In the middle region of the stomach, the kidney follicles lie above the left mantle and the outer hemibranch of the left gill. As the follicles of the kidney extend inwards they are accompanied by the ducts of the gonad, which lie in the vesicular connective tissue between the follicles of the kidney and the digestive diverticula. The ducts of the gonad now vary very greatly in cross section; some are rounded in outline, while others are oval or very elongated.

Toward the posterior extremity of the stomach, the ducts of the gonad begin to invade the vesicular tissue above the mantle and gills on the right side; they extend as far as the right outer hemibranch. On the left side they now extend as

far as the left inner hemibranch; they are closely followed by the follicles of the left kidney.

The follicles of the right kidney appear above the right outer and inner hemibranchs of the gills in a vertical plane which traverses the anterior extremity of the mid-gut and style sac; a follicle also appears now on the right side slightly inferior to the right mantle on the dorso-lateral surface and external to the ducts of the gonad; it is quickly followed by branches which extend about half-way between the attachments of the right and left mantles dorsally and also on the right side between the surface epithelium and the ducts of the gonad. Back slightly farther they form an almost complete ring of follicles on the right side extending from the dorsal aorta, midway between the attachment of the right and left mantles dorsally, to above the attachment of the right outer hemibranch. Dorsally, also, the ducts of the gonad curve inwards on both sides to lie below the dorsal aorta and the hind-gut as it approaches the pericardium. Here, the digestive diverticula occur only on the right and left sides of the mid-gut and style sac; they no longer surround them above and below. On the left side the follicles of the kidney now form a double layer. The ducts of the gonad continue to penetrate the vesicular connective tissue above the attachment of the gills, and in this region almost meet in the middle line.

In the region where the sections cut through the front of the pericardium towards its dorsal aspect, a very different scene is presented. Dorsally we find the rectum bounded laterally by the dorsal extensions of the mantle; below the rectum lies the pericardium containing the ventricle. The follicles of the kidney fill up in several layers the greatly thickened right wall of the pericardium and extend below it in a single layer immediately below the surface epithelium to above the right inner hemibranch of the gills. On the left side the wall of the pericardium is extremely thin and no kidney follicles extend into it; they begin dorsally immediately beneath the attachment of the left wall of the pericardium to the oral process and extend down the lateral border of the oral process to above the left inner hemibranch of the gills; they are particularly numerous in the triangular area bounded on the left side by the surface epithelium, below by the mantle and the outer and inner hemibranchs, and in the right dorso-lateral region by the ducts of the gonad; they do not extend here into the dorsal region of the oral process, below the pericardium. The ducts of the gonad form an almost continuous layer along the dorsal surface of the oral process, below the epithelium lining the pericardium ventrally, and on each side of the oral process just internal to the kidney follicles. The mid-gut and style sac take up a considerable space in the centre of the sections; they are surrounded on the right and left sides by digestive diverticula, which lie scattered in the vesicular connective tissue and partially surround the loop of the intestine returning from the oral process.

This disposition of the various organs continues till about midway beneath the pericardium when the digestive diverticula no longer appear, the mid-gut and accompanying style sac then being separated from the ducts of the gonad by a thick layer of vesicular tissue only, except on the right side, which is traversed by the returning loop of the intestine. Here also the right wall of the pericardium contains but one extremely large kidney follicle; while on the left side a similar large follicle has developed in the triangular area described above. These follicles comprise what may be regarded as urinary chambers (right and left).

Below the posterior region of the pericardium and the auricles, the follicles of the kidney again extend from each side into the dorsal surface of the oral process, immediately beneath the pericardium, and are separated from the pericardium by a thin membrane; they no longer extend into the ventral surface of the oral process above the gills; they occur in greatest abundance and are largest in the right wall of the pericardium, while on the left side they comprise the bulk of the tissues between the style sac and the surface of the body bounded above by the pericardium and below by the mantle and the left outer hemibranch of the gills. The ducts of the gonad lie very close to the internal margins of the kidney follicles, and these, too, no longer extend into the area above the attachment of the gills.

In sections cut through the posterior extremity of the pericardium, which is here reduced to a relatively small cavity below the adductor muscle, the kidney on the right side increases very greatly in extent; the urinary chamber now lies in two planes like a letter L, the vertical arm extending from the adductor muscle dorsally to immediately above the branchial cavity ventrally; while the horizontal arm lies in the pericardium above the dorsal surface of the oral process, from which it is separated by a layer of large kidney follicles. The left kidney is here much smaller; the urinary chamber, irregularly oval in shape, with the long axis vertical, lies in close apposition with the left wall of the oral process, while follicles extend from its dorsal surface along the upper wall of the oral process to meet those of the right kidney. Externally it is bounded by kidney follicles which separate it from the surface epithelium above the attachment of the left mantle. The ducts of the gonad are now small and few in number; they lie beneath the dorsal surface of the oral process and extend on each side to the branchial cavity.

It is in this region that the gonoduct of the right side opens into the urino-genital cleft (Pl. xiii, fig. 8, *u.g.c.*). The duct lying nearest to the lateral wall of the oral process is much larger than the rest; as it approaches the urino-genital cleft it is crescent-shaped, the convex wall external and its surface strongly ciliated, particularly in the region nearest the branchial cavity, while the internal wall is lined by germinal epithelium and lies in close apposition with a prominent nerve, the right visceral nerve (*r.v.n.*). The gonoducts of both sides are surrounded by bands of sphincter muscle fibres immediately internal to the cleft, and are lined by ciliated epithelium. The gonoduct of the right side runs in a dorso-ventral direction; it is situated at the side of and slightly above the returning loop of the intestine, which lies very close to the right wall of the oral process, the mid-gut and style sac occupying the central area.

Slightly farther back the urinary chamber empties, by means of a short canal, the ureter, into the urino-genital cleft. The ureter also runs in a dorso-ventral direction, and is simply a ventral extension of the urinary chamber. The ureter therefore opens slightly posterior to the gonoduct, and slightly to its right in a somewhat higher plane; it is lined by ciliated epithelium, the cilia being long and very numerous and filling up the lumen of the canal. The lower portion of the urinary chamber is also lined by strongly ciliated epithelium, and is surrounded by a narrow band of muscles which extends to the ureter and encloses it on all sides. The right visceral nerve, which lay in close contact with the genital duct, here lies internal to and below the wall of the ureter, while immediately external to the wall of the urinary chamber, a little above the ureter, lies the larger right branchial nerve (*b.n.*).

The gonoduct on the left side opens into the urino-genital cleft (Pl. xiii, fig. 9, *u.g.c.*) slightly posterior to that of the right side; it crosses the median line of the oral process and, like that of the right side, extends in a dorso-ventral direction. Its internal surface is in close contact with the left visceral nerve (*l.v.n.*).

A little farther back again the ureter opens downwards into the urino-genital cleft. The urinary chamber on the left side lies alongside the oral process, and not dorsal to it as on the right side. The urino-genital cleft is bounded internally by the epithelium of the oral process which is here ciliated, and externally by similar ciliated epithelium which is separated from that of the urinary chamber by a thin layer of connective tissue. As on the right side, the walls of the urinary chamber and ureter are surrounded by a thin band of muscle fibres. Immediately above the ureter the left visceral nerve runs in an antero-posterior direction. Ventrally the wall of the urinary chamber is traversed by the large left branchial nerve (*b.n.*) which causes a pronounced dilatation of the wall. The epithelium of the urinary chamber is lined by cilia which become very long and numerous in the vicinity of the ureter.

The ducts of the gonad lying just beneath the dorsal surface of the oral process extend a little farther back than the openings of the gonoducts on each side, and terminate at about the level of the ureter.

In the plane of the left ureter the follicles of the kidney form a thick layer between the adductor muscle and the dorsal surface of the oral process along its whole length; on the right side they do not extend below this level; on the left side, however, they continue downwards on the side of the oral process as far as the opening of the ureter.

Continuing posteriorly, the kidney follicles extend beyond the termination of the oral process to about the posterior surfaces of the visceral ganglia.

The Development of the Gonad.

In the very young oyster, in which the sexual products are developing for the first time, the layer of vesicular connective tissue surrounding the digestive diverticula is of very irregular thickness; in places the digestive diverticula may extend almost to the surface epithelium, while in others the intervening layer of vesicular tissue may be of considerable thickness, but nowhere does it attain the great depth characteristic of an older oyster which is about to develop sexual products at the beginning of the summer. In this young stage all oysters from which sections have been prepared were males, and it is, I think, very unlikely that the development of the eggs can be studied in an oyster of this species which has not previously developed spermatozoa. Just beneath the surface epithelium, and separated from it by a layer of vesicular tissue from one to three or four cells deep, the ducts of the gonad, mostly elongated in cross section and lenticular in shape but occasionally smaller and broadly oval, extend in a band round the oyster. These ducts are lined along their outer surfaces by ciliated epithelium, and along their inner surfaces by germinal epithelium which, in the youngest oysters of which I have prepared sections, is already giving rise to spermatogonia; these, however, have not yet begun to produce spermatocytes. Irregularly distributed amongst the small, flattened cells of the germinal epithelium are some, the spermatogonia, which have become relatively greatly swollen and contain a large nucleus with a great number of chromatin granules. These spermatogonia are

irregular in outline and variable in size, the largest being 14μ in diameter and their nuclei 10μ.

In slightly older oysters proliferation of the spermatogonia into spermatocytes and development of the latter into spermatozoa can be clearly distinguished (Pl. xiv, figs. 10, 11). The primary spermatocytes, or spermatocytes of the first order (Pl. xiv, fig. 10, *sp.*[1]), form a layer immediately above the spermatogonia (*spg.*); they are rounded in shape and measure up to 6μ in diameter; the nucleus occupies the bulk of the cell, and its chromatin granules, although numerous and deeply stained, are somewhat scattered, and give to the nucleus a general appearance of being lightly stained. Immediately above the primary spermatocytes, the secondary spermatocytes, or spermatocytes of the second order (*sp.*[2]) lie in a somewhat deeper layer; these measure up to 3·5μ; the nucleus is large, and the chromatin more concentrated, hence they appear to be more deeply stained. Above the secondary spermatocytes, and almost completely filling the lumen of the duct, are the spermatids and spermatozoa (*s.*). The heads of the latter measure 1·5μ in diameter (in sections), and their chromatin, now extremely concentrated, stains very deeply. The tails lie in bands between the lanes of spermatozoa, and always point towards the outer surface of the duct.

As development of the testis proceeds, the vesicular connective tissue, both external and internal to the genital ducts, increases rapidly till it forms a thick layer separating them from the surface epithelium externally and from the digestive diverticula internally. When the ducts are distended with spermatozoa, offshoots begin to sink into the vesicular tissue which borders them internally. These may extend in irregular follicles as far as the digestive diverticula, or they may give off branches which, running more or less horizontally, are cut across in sections made at right angles to the surface of the gonad (Pl. xv, fig. 12). Germinal epithelium usually lines the walls of these follicles, from which spermatogonia, spermatocytes and spermatozoa continue to develop. Nourishment for the developing sex cells is obtained from the vesicular tissue, and as proliferation proceeds the ducts and follicles tend to coalesce with the partial or even total disappearance of the follicular walls, until, as the testis approaches maturity, they may almost cease to exist as separate entities.

In a male oyster just prior to spawning (Pl. xv, fig. 13), the vesicular tissue lying between the genital ducts and the surface epithelium may become reduced to a narrow band, and in places may disappear entirely; the ducts in cross section are of great width and are divided from one another by narrow bands of vesicular tissue which extend downwards into the testis for varying distances, becoming narrower as they proceed, while the spermatozoa lie in intimate contact with the ciliated epithelium of the ducts. The testis rarely becomes a uniform mass of spermatozoa, but is broken up into irregular islands by small areas of vesicular tissue, which are frequently bordered by spermatocytes. In these areas of vesicular tissue an occasional blood space is to be found. The tails of the spermatozoa are usually bunched together in definite streams, which run in all directions.

The head of the live sperm of *O. commercialis* measures 2μ in diameter, and the tail 42μ long. The shape of the head of this sperm differs from that of *O. virginica*, which is described by Stafford (1913) as "almost oval in form, somewhat pointed anteriorly, but inclined to be squarish posteriorly". The head of the sperm of *O. commercialis* is spherical (Text-fig. 2). Attached to the head, and grouped round the insertion of the tail are four spherules, of which usually only two, occasionally three, can be seen when the sperm is lying on its side.

It need scarcely be remarked that sperm morulae, characteristic of larviparous oysters, do not occur in *O. commercialis*.

The head of the sperm of the American oyster, *O. virginica*, is stated by Stafford (1913) to measure 1·75μ in breadth, and the tail 35μ in length; while Churchill (1920) states that the head measures 2·5μ, and the tail about 50μ; that of *O. edulis* is stated by Hoek (1883) to be at most 1μ, and the tail 25μ, while Orton in the Encyclopaedia Britannica (14th Edition) gives the measurement of the head as 2μ, and the tail about 20–30μ.

When considering the development of the ovary, it should be borne in mind that we are dealing with an oyster which has probably already functioned as a male. In all young females which I have examined by means of microscopic sections, the layer of vesicular connective tissue in which the germinal ducts lie is of far greater depth than in young males in which the sex organ is developing for the first time. This vesicular tissue (Pl. xvi, fig. 14, *v.t.*) forms a thick layer between the ducts and the surface epithelium, and also between them and the digestive diverticula. There is, therefore, already awaiting the development of the eggs, which require more nutriment for their growth than the sperms, an abundance of vesicular tissue in which nutriment, mainly in the form of glycogen, is stored.

External to the genital ducts the layer of vesicular tissue is usually homogeneous and of rather loose texture; occasionally, however, patches occur in which the tissue becomes very condensed, the cells losing their characteristic form of bladder cells and becoming smaller and more compact. Similar condensed areas may occur in the broader band of vesicular tissue lying internal to the ducts; in the latter, numerous blood spaces occur, the tissue bordering them being usually condensed.

The germinal ducts (Pl. xvi, figs. 14, 15, *d.*, *d.*[1]) are for the most part lenticular in cross section, but oval or circular ducts are not uncommon. The distance between the ducts varies greatly; in some cases a band of condensed vesicular tissue of variable width extends from one duct to the next; in others, it may continue along the external surfaces of the ducts and form a more or less unbroken band bordering them externally (Pl. xxii, fig. 26, *v.t.*[3]); in other cases, again, no condensation at all may occur in the tissue between or external to the ducts. Muscle fibres are frequently present in this band of tissue, particularly in that portion of it in contact with the ciliated epithelium of the ducts.

As in the testis, the ducts are lined by ciliated epithelium externally (Pl. xvi, fig. 15, Pl. xvii, fig. 16, *c.e.*), and internally by germinal epithelium, from which the ova develop. In their very earliest stages it is extremely difficult, if not impossible, to distinguish the male from the female germ cells. There is usually no difficulty, however, in determining the sex of the individual, for easily recognizable young ova early become apparent in some of the ducts; they are characterized by their granular cytoplasm, large, comparatively clear nucleus, and conspicuous, deeply-staining nucleolus. As they develop they become enormously swollen, attached to the basement membrane by a broad base, and project into the lumen of the duct as a dome-shaped cell, the nucleus lying close to the attached surface. As they continue to grow, the development of other ova at their bases compresses the region of their attachment till it becomes constricted into a long, narrow neck, the free portion remaining swollen, and the whole resembling an attenuated pear (Pl. xvii, fig. 16, *o.*). The nucleus now lies in the centre of the swollen extremity, with the nucleolus close to its circumference.

With the crowding of the germinal layer by the rapid development of the eggs, portion or portions of it evaginate into the vesicular tissue, and, by further evaginations, form numerous branching and anastomosing follicles (Pl. xvi, figs. 14, 15, *f.*). As the ova in these follicles develop, they are continually absorbing nourishment from the vesicular tissue, which rapidly diminishes in extent, till in the ripe ovary, none, or practically none, remains. The mature ovary, therefore, consists of a mass of closely packed ova attached to the follicular walls by their narrow extremities (Pl. xvii, figs. 16, 17, *o.*). The germinal ducts, in the meantime, have become greatly extended laterally; many have joined up with their neighbours, whilst others are separated only by a narrow band of vesicular tissue or by the walls of the follicles. The layer of vesicular tissue lying between the ducts and the surface of the oyster also diminishes rapidly as the eggs develop, till in the mature ovary it becomes reduced to a very narrow layer (Pl. xvii, fig. 16).

Conspicuous amongst the cells of the ciliated epithelium of the germinal ducts of both the ovary and testis are frequently to be seen granular cells which stain very deeply with eosin and other acid stains. These cells may be relatively small, no larger than the epithelial cells, but more frequently are greatly swollen and project well into the lumen of the duct (Pl. xiv, fig. 11; Pl. xvi, fig. 15; Pl. xxiii, fig. 28, *s.c.*). Occasionally neighbouring cells may coalesce to form a continuous layer of considerable extent. Some ducts may be entirely devoid of them, whilst in others the ciliated epithelium may be entirely replaced by them. They may be seen at times extruding their granular contents into the lumen of the duct. Whilst these granular cells are usually devoid of cilia, occasionally a few cilia may be seen projecting from the surface; they appear, therefore, to have originated as ordinary epithelial cells, later differentiating to secrete granular material. They occur in the ducts of the gonad in all stages of its development, from the time when the sex cells are beginning to differentiate, till the gonad is mature. They reach their greatest development, however, and usually occur in greatest abundance, in partially spawned or completely spawned oysters (Pl. xviii, fig. 19; Pl. xix, fig. 21; Pl. xxi, fig. 24, *s.c.*); in several successive ducts they may completely replace the ciliated epithelium, and form a layer from one to several cells deep, and may even fill almost the whole of the lumen of the duct. But in an oyster in which this excessive development occurs, a few of the ducts may remain almost, or even entirely, devoid of them. Such prolific development, however, is not to be found in all recently spawned oysters, for occasionally one will be found in which the granular cells occur in very few of the ducts, and are inconspicuous and sparsely distributed.

The function of these cells I have been unable to determine. They are not mucous cells, mucicarmine having no affinity for them whatever. They stain intensely with eosin, erythrosin, acid fuchsin and iron haematoxylin, and very slightly with Delafield's and Ehrlich's haematoxylin. In preparations stained with iron haematoxylin and acid fuchsin, the granules in some of the cells are rendered a deep bluish-black, whilst in others they are a brilliant red. In eosin-methylene blue preparations they form a beautiful contrast.

Granular cells with similar staining characteristics are found in the surface epithelium, where at times they occur in great abundance and may even form an almost continuous layer of considerable extent, and in that of the intestine. In the latter situation they have been noted by several authors in various molluscs,

and have usually been designated "eosinophilous cells" from their great affinity for this stain.

Mature eggs of the oyster may be studied either alive or in sections; in the former case, they may be obtained by pricking the surface of a ripe ovary, or preferably by a little gentle pressure of the finger along the surface of the ovary from before backwards, thereby forcing some of the eggs out of the oviduct. The eggs of *O. commercialis* (Pl. xviii, fig. 18) may be round, oval, or more commonly pear-shaped; if the latter, the narrow end may be greatly prolonged. They vary considerably in size; the width of pear-shaped eggs varies usually from 0·05 to 0·06 mm., while the longest I have seen measured 0·225 mm. The largest eggs I have measured were subspherical, the diameter being 0·09 mm. The shape of the egg is not an indication of maturity, for an elongated pear-shaped egg is as capable of fertilization and subsequent development as a spherical one. Soon after fertilization, however, an elongated egg becomes spherical. In the Bombay oyster (*O. cucullata*), Awati and Rai (1931) found that the spherical eggs alone were ripe and capable of fertilization.

The eggs of all oviparous oysters appear to average approximately the same size, i.e., 0·05 mm., as stated by Stafford (1913), Churchill (1920), and T. C. Nelson (1921) for the American *O. virginica*; Amemiya (1928) for the Japanese *O. gigas*, *O. spinosa*, and *O. circumspicta*; and Amemiya (1926) for the Portuguese *O. angulata*. The eggs of the larviparous types are all larger and measure 0·1 mm., as stated by Amemiya (1928) for the European *O. edulis* and the Japanese *O. denselamellosa*; and Stafford (1913*a*) for the American *O. lurida*.

The eggs are enclosed in a very thin membrane; they contain granular cytoplasm with a narrow, clear, homogeneous band immediately beneath the lining membrane, and another surrounding the nucleus. The nucleus is very large and spherical in shape; it contains granular nucleoplasm more transparent than the cytoplasm surrounding it. The nucleolus appears as a small glistening spherical object, which usually lies in close apposition with the nuclear membrane, but may occur anywhere in its interior.

In sections stained with haematoxylin the granules of the cytoplasm, the nuclear membrane, and particularly the nucleolus, stain deeply, while the protoplasm of the nucleus stains lightly and tends to become reticulated (Pl. xvii, fig. 17, *o.*).

The Gonad during and after Spawning.

When an oyster spawns, the reproductive elements, ova or sperms, are conducted along the ciliated ducts to the gonoducts on each side, which, as we have seen, open on the sides of the oral process beneath the adductor muscle. As batches of eggs or sperms are swept along these ducts, further batches are drawn upwards, where they enter the stream formed by the beating of the cilia. In this way the follicles are gradually drained.

Although I have prepared sections of many oysters during and after the spawning period (during late summer and winter), I have not found one in which spawning has been complete, i.e., in which the whole of the eggs or sperms have been ejected. It is apparently usual for some to remain in the gonad; indeed, at times the bulk of them are retained, and individuals are occasionally found in batches of recently spawned oysters which have obviously not spawned at all. In the latter case the eggs or sperms may either be carried over the winter or, as appears to be more frequent, they may be absorbed.

Absorption of the undiscarded eggs or sperms of a partially spawned oyster appears always to take place; the process is a comparatively slow one, for various stages of absorption may be seen during the whole of the winter following spawning.

In the case of the testis, the ducts and follicles containing spermatozoa become infiltrated with small, irregular, densely-crowded, vesicular cells (Pl. xviii, fig. 19, *v.t.*^{1}, *v.t.*^{2}). These cells are in very active division and mitoses are greatly in evidence. Gradually, as the spermatozoa are absorbed, they are completely replaced by the condensed vesicular cells (Pl. xix, fig. 20, *v.t.*^{1}, *v.t.*^{2}), which continue to develop and expand until, early in the following spring, they assume the typical bladder-like character of the ordinary vesicular cells characteristic of this region. Their function is, of course, to store nutriment for the ensuing development of spermatozoa or, possibly, eggs.

In the case of the ovary, absorption of undischarged eggs takes place in a similar manner. A few small vesicular cells make their appearance amongst the eggs in the ducts and follicles, and develop at the expense of the eggs (Pl. xix, fig. 21; Pl. xx, fig. 22, *o.*, *o.*^{1}, *v.t.*^{1}, *v.t.*^{2}). Gradually, as the vesicular cells increase in numbers, the eggs degenerate, decrease in size, and eventually disappear altogether, their place being taken by densely crowded vesicular cells (Pl. xx, fig. 23; Pl. xxi, fig. 24, *v.t.*^{1}, *v.t.*^{2}). When absorption is complete the vesicular cells continue their growth by extracting nourishment from the blood stream, until the whole of this region comes to consist of large, more or less uniform, vesicular or bladder cells which characterized it before the sex cells began their development.

As the genital products are absorbed, the ducts shrink away from their neighbours with which they had coalesced as the gonad reached an advanced stage of development. They remain filled for a considerable time, however, with the condensed vesicular cells (Pl. xxi, fig. 25, *v.t.*^{1}) which have replaced the eggs. Gradually these cells migrate downwards until the ducts are completely free from them; the ducts then again assume the form they had when the sex cells began to differentiate, i.e., they are lined by germinal epithelium internally and ciliated epithelium externally.

Considerable difficulty was encountered in the preparation of sections of recently spawned oysters. The difficulty was apparently one of fixation, for the layer of vesicular connective tissue in which the sex cells were embedded, and sometimes also the layer between the genital ducts and the surface epithelium, was usually found to undergo considerable disintegration during spawning. The fixatives used consisted of Bouin's picro-formol-acetic, Zenker-formol (Helly's fluid), and Carnoy's acetic-alcohol-chloroform mixture. None of them was found to be satisfactory. For general histological work on the oyster, I have found Bouin's fluid to be a satisfactory fixative; Zenker-formol was found to distort some of the tissues badly; and Carnoy's fluid, excellent for some tissues, was found very poor for others; it usually caused a shrinkage of the ducts and the breaking away of the cilia from the epithelium. In my earlier histological work most of the tissues were fixed in formol-alcohol-acetic of Bles, with fairly satisfactory results.

The Spawning of O. commercialis.

My observations on the spawning habits of *O. commercialis* were made at Port Macquarie, a harbour at the entrance of the Hastings River, on the north coast of New South Wales. Port Macquarie is a large basin which receives the water from the Hastings River at its north-east corner, and has a narrow outlet to the Pacific

in the south-east quarter through which the tidal waters reach the sea. Owing to the narrowness and shallowness of this channel, the rise and fall of the tide in the estuary is never very great. The channel itself is bounded along its southern border by a breakwater, and requires frequent dredging to allow of the passage of craft of even shallow draught. The harbour has two channels, the eastern being navigable by steamers of small draught, while the western is impassable by ocean-going vessels of any description, the central area consisting of a large sand spit which is bared by the tide.

Oysters are cultivated along the whole of the western foreshores to within about half a mile of the breakwater. Choice of this locality was made because the water is usually very clear, allowing of unobscured observation of the oysters when submerged, and also because the actual spawning is usually heavy, and abundant catches of spat are a general rule. Owing to the proximity to the ocean the density of the water is usually rather high, though a good deal of fresh water spills over from a large swamp inshore after heavy rain.

Having received, about the middle of February, 1924, some oysters from Port Macquarie which had the reproductive organ in a very advanced state of development, containing an abundance of ripe ova and sperms, I proceeded there on 25th February and made a daily survey of the beds in an endeavour to discover any indications of spawning oysters; in this I was assisted by the local Inspector of Fisheries and by the oyster growers, who were working the beds continuously. Although the oysters everywhere were very fat, no spawning was seen until 5th April, and although numbers were repeatedly opened for examination, none previous to this date had given any indication of even a partial spawning. In the meantime a plankton net consisting of No. 16 bolting silk (157 meshes to the inch) was towed over the beds daily, in many situations, at different states of the tide, and from the surface of the water to close to the bottom, but in no instance was an oyster larva seen, although the larvae of many other bivalves were always abundant (Pl. xxiv, fig. 30).

On 5th April, the late Mr. Thomas Dick, a prominent oyster grower of the district, noticed oysters beginning to spawn a short distance from where my laboratory was situated. Hastening to the bed, I found that the water over it was distinctly cloudy, and in a very short time the bottom and the stones to which the oysters were attached were obscured from view in water two feet deep. Spawning had begun about 12.30 p.m.; the temperature of the water at the surface was 70° F., and at the bottom 68° F.; the density at the surface was 1·0195, and at the bottom 1·020; the tide, which had been a particularly high spring tide (new moon) was from three to four hours on the *ebb*, and when the oysters began spawning was about mean high water level. The wind was moderately strong from the south, though the beds were fairly sheltered by a lee shore; the tide was ebbing very slowly, a heavy sea outside the bar tending to bank the water in. Heavy rain had fallen during the previous day, but the density of the water was little affected by it. By 1.30 p.m. (an hour after the commencement of spawning), the water had practically cleared, and although the beds were patrolled for about a quarter of a mile, no evidence of spawning could be seen beyond the bed under observation, which covered an area of about a hundred yards long by about twenty wide.

The late Mr. Thomas Dick, whose observations in the realm of oyster life were particularly keen and very reliable, informed me that spawning invariably occurs on these beds during spring tides when two or three hours on the ebb, and often

when a heavy sea is running outside. On one occasion previously he had taken the temperature of the surface water over a shallow bed and found it to be 70° F.

The temperature of the water over the beds had been taken daily from 25th February till spawning occurred on 5th April, and on only one occasion was it found below 70° F., when on the day preceding spawning it was 68° F. It varied from 70° F. to 85° F., and was usually from 72° F. to 76° F. The oysters during the whole of this time appeared to be capable of spawning, and fertilization was always readily effected when ova and sperms were mixed in sea water. It would appear, therefore, that other physical conditions besides temperature play a part in governing the spawning impulse.

There is abundant evidence that the heaviest spawnings occur on the New South Wales coast at spring tides (both full and new moon tidal periods), and cultch is frequently laid out just before Christmas, when a heavy spawning often occurs during the unusually high tides which occur at that period. But if spawning were confined to spring tides the resulting sets of spat would assuredly occur in well defined fortnightly periods, for, even allowing for irregularities of growth, the bulk of the larvae may be expected to develop with a certain amount of uniformity. An investigation during three months on the Hawkesbury River in 1925, however, showed that this, at least in that river, is not always the case. The attachment of spat occurred with the utmost irregularity; practically every day a few spat were found attached to shells, fibro-cement sheets, glass, and other objects placed out to secure them. On some days more would attach than on others, but there was never a heavy set during any period of twenty-four hours. In the aggregate, the catch was a good one, but it extended over a period of five months. It appears to be a fair assumption that spawning in various localities was proceeding daily or almost daily during the whole of that period. Such conditions appear to be normal to the Hawkesbury River; inquiry amongst the most experienced cultivators showed that none had ever seen the water rendered milky by oyster spawn, and few indeed had ever seen a spawning oyster. Apparently it is customary for the oysters to spawn partially at intervals, and in this respect they are in marked contrast with those at Port Macquarie, where a general and heavy spawning may occur during the course of one tide, resulting in an almost simultaneous set of spat. I have seen fibro-cement sheets from Port Macquarie with an enormously heavy set of very young spat which had every appearance of being of a uniform age. Oyster growers there have seen the water clouded with spawn for hundreds of yards.

Further observations of the spawning of the oysters on 5th April, 1924, showed that two methods were commonly employed to expel the genital products: one, the more common, being a forcible ejection, the spawn in this case leaving that part of the shell situated closest to the gonoduct, i.e., directly inferior to the adductor muscle; and the other, a mere dribble, when the spawn usually oozed from the shells in the exhalant current at the dorsal surface, above and behind the adductor muscle; less frequently it would ooze from the same region (inferiorly) as that from which it was forcibly ejected.

The forcible ejection was accomplished by a gradual opening of the shells, followed by a sudden closure, accompanied, when out of water, by a distinct hissing sound, the spawn leaving the shells in a cloud, which resembled the form of the smoke emitted when a cannon is fired. Immediately after leaving the shell it was concentrated in a thin, swiftly-moving stream, but gradually became more diffuse

and slowly dispersed into the water. The intervals between each ejectment were of short and irregular duration during the early stages, but gradually became longer as the process was continued; the force of the expulsion was also greater at the beginning than towards the end.

It has been stated by T. C. Nelson (1921) that the spawn of the American oyster oozes from the gaping shells of the males, while the females forcibly eject it at intervals, and a similar observation was made by Prytherch (1923). At Port Macquarie no such rigid differentiation could be made, for sperms were occasionally seen to be forcibly ejected from the male oysters, and eggs sometimes to trickle from the females, in each case from the ventral margin of the shell. Observations were made on specimens which were placed singly in glass jars, and verification in each case was obtained by means of microscopical examination. It was also seen that the spawn may be forcibly ejected or ooze out of the same oyster, though the exudation in this case was only seen after the oyster had been spawning for some time. After one ejection the shells remain closed for some time; they then slowly open very widely, as much as a quarter of an inch, when the sudden snapping of the shells is repeated.

A stone with oysters attached was removed from about two feet of water to shallower water inshore where portion only of the stone was submerged; here the oysters both in the water and exposed to the air continued to spawn; in the latter case one squirted the fluid into a beaker held at the level of the oyster about twelve inches distant.

A marked contrast was noted between the dispersal of the eggs and that of the sperms. The eggs quickly diffused, whereas the sperms were inclined to hold together for a short time as well defined white streaks. Numbers of oysters, having begun to spawn in the water, were lifted into the boat, and in almost every case they continued to spawn at intervals similar to those remaining in the water. Several, having completed spawning, remained with the shells gaping widely; these were left in the boat and two days afterwards were found to be dead, although normally this species of oyster will live for upwards of two weeks out of water. The exhaustion of spawning appeared to render them utterly unable to effect the closure of the shells, and in the absence of a normal flow of water, they quickly perished. Several of the gaping oysters were opened and in most cases it was found that about half the contents of the gonad had been drained. The extent to which spawning continues appears to regulate the degree of exhaustion.

Having begun to spawn, the oyster will continue the process in spite of rough handling. For instance, some oysters which had been seen to eject spawn were forcibly knocked, by means of a culling iron, from the stone to which they were securely attached, and when placed in a jar of water soon opened their shells and continued to eject the spawn.

If spawning occurs on a bed at a time when they are being gathered for market, it is necessary to return all the oysters to the bed, for they may continue to spawn in the bag and die before they reach the market.

A marked difference was noticed in the nature of the fluid ejected from the oysters at the beginning of spawning and when it was nearing completion; in the former case it was more uniform and dispersed more rapidly, while in the latter, it was accompanied by relatively large pieces of tissue, apparently from the broken-down gonad.

The eggs and sperms quickly settle to the bottom. In a large beaker into which four spawning oysters were placed, the water was for a time quite milky and

opaque, and the outline of the oysters could not be seen when looked at from above; settlement was proceeding the whole time, the water at the bottom of the beaker becoming increasingly opaque, while that at the surface became gradually clearer. At the end of half an hour the water was perfectly clear, and the spawn lay in a dense layer on the bottom of the glass and on the surface of the oysters like a mantle of snow.

In an endeavour to procure as much information as possible relative to the state of the tide when *O. commercialis* spawns, I called for reports (through the courtesy of State Fisheries) from the Inspectors of Fisheries stationed along the New South Wales coast. These Inspectors are continually moving about the oyster leases, and have a splendid opportunity of observing the condition of the oysters. Altogether, 22 instances of spawning were reported, and of these 15 were to the effect that spawning occurred on the ebb tide, and 7 on the flood. Eleven spawnings were recorded during spring tides, and four during neap tides; in seven of the spawnings reported no note had been taken of the tidal range.

Three of these reports drew attention to the fact that young mullet (*Mugil* sp.) were attracted to the spawning, and by their activity, they gave every indication of devouring large quantities of spawn.

In two instances the Inspectors stated that they had seen the spawning continue after the oysters were bared by the tide, leaving the shells more or less covered with the white fluid.

For purposes of comparison it will be of interest to survey the observations of the spawning of different species recorded by various authors in other parts of the world.

Orton (1920) states that, throughout its range, the larviparous European oyster (*O. edulis*) appears to begin to breed at a temperature of about 59° F.–61° F. in varying salinities from about 25‰ to as high as 36‰ or more in the Gulf of Naples, and continues to breed so long as the temperature remains above this figure, so that in the warmer situations there is a longer breeding period than in the colder ones.

Spärck (1925) found, however, that the breeding temperature varies according to whether the spring rise in temperature is rapid or slow. The breeding temperature was found to be higher the more steeply the temperature curve rises. After a warm spring, oysters were found with larvae in the mantle cavity at a temperature of 55° F.–57° F.; after a cold spring, when the temperature of the water rose rapidly, no oysters with developing larvae were found till a temperature of 61° F.–63° F. was reached.

A lunar periodicity has been stated by Orton (1926) to occur in *O. edulis*, a maximum of spawning being found round about the days of full moon. It is suggested that the rapid rate of change in pressure accompanied by increased temperature at spring tides may be sufficient stimulus to cause spawning to occur. In the Fal estuary in 1926, Orton (1927*a*) found that regular spawning began on the spring tides at about full moon at the end of June, and continued throughout the summer until the full moon spring tides about 23rd September.

In the American oyster (*O. virginica*), J. Nelson (1890) found that in New Jersey spawning begins when the temperature of the water reaches 70° F.

Stafford (1913) found that in Canadian waters the temperature of the water in the region of oyster beds at the beginning of spawning approximates to 68° F.

Churchill and Gutsell (1919) state that in Great South Bay, Long Island, spawning began when the temperature of the water reached 70° F., and that it proceeded rapidly while temperatures ranged from 70° to 74°, but that it slowed down or ceased with temperatures of 70° to 68° F. When it rose to 74° F. the bulk of the spawn was thrown out in the course of two or three days. In the same locality Gutsell (1921) found that in 1921 spawning began with a water temperature of 66·5° F. to 67° F.

Churchill (1920) states that *O. virginica* may spawn when the water reaches a temperature of 68° F., but spawning proceeds at normal speed only when the water is 70° or above.

T. C. Nelson (1921) found that, although spawning of oysters will commence when the water has reached a temperature of about 70° F., it becomes much more active with temperatures from 75° F. to 85° F. A rise in temperature is followed by a more active spawning, while a decrease in temperature is accompanied by a rapid falling off in the amount liberated.

Spawning was observed by Nelson on a float some six inches below the surface of the water on 26th June, 1920, at 4.55 p.m., in bright sunlight. The tide was about three-quarter flood, the density 1·018, and the temperature 78° F. Spawning continued till 5 p.m. About one-third of the spawn was thrown out during this spawning. An examination of the stomachs of the oysters showed that the oysters had not been feeding during the duration of the spawning.

T. C. Nelson (1921*a*) reproduced a kymograph tracing made by two female oysters which spawned while attached to an apparatus used to record feeding reactions. The first oyster spawned on 6th August, 1920, from 4.18 p.m. to 4.29 p.m. with the tide five-sixths flood, and the second on 26th July at 10.37 p.m. at high tide.

Prytherch (1923) found that by placing oysters in tanks of slowly running water, in the sunlight, spawning may be induced by the rising temperature. In every such experiment from 10th July to 1st September the oysters spawned readily. Spawning occurred at temperatures ranging from 68° F. to 75° F., and lasted over intervals of 15 to 30 minutes. At temperatures higher than 75° F., it was found that the spawn was released slowly and immediately settled to the bottom of the tank, whereas normally it would be forcibly ejected into the water and would float about for some time. In one instance a group of 11 months old oysters spawned for over five minutes at a temperature of 69·5° F. A sample of this, observed in a watch-glass, developed as normally as that of the older oysters.

T. C. Nelson (1925) found a latent period of four days after the temperature rose above 68° F. before spawning began.

The same author (1926) confirmed J. Nelson's observations that after the spawning temperature (68° F.) has been attained a sharp rise in temperature is followed by spawning. He also found that spawning is most apt to occur between late afternoon and midnight, and during the flood tide. T. C. Nelson observed or recorded on a kymograph natural spawning of the oyster on five occasions. In each instance the spawning took place in the daytime during the late flood tide and with a rising temperature.

Galtsoff (1926) found that both male and female oysters may be induced to spawn by increasing the temperature of the water, whereas spawning in the female may be induced at a constant temperature by the addition of sperm. After spawning, a female oyster is for two days immune to the presence of sperm.

T. C. Nelson (1927) observed that the more rapid the rise in temperature above 68° F. the sooner spawning begins, though a higher temperature is required to induce spawning than when the temperature rise has been more gradual and extended over a longer time. In the latter respect Nelson's observations on the spawning of *O. virginica* are in entire agreement with those of Spärck (1925) on *O. edulis*.

T. C. Nelson (1928*a*) stated that later spawnings occur usually at temperatures above that which caused the initial spawning of the season. Following a cold summer, unshed spawn may be found in the oysters even into the winter months. The important observation was made, also, that the American oyster spawns after the water temperature reaches 68° F. over all parts of its range, with no adjustment to the extremes of its distribution.

Prytherch (1928) states that in many instances in 1925 and 1926 spawning was observed to take place in tanks and floats (in Milford Harbour, Connecticut) at temperatures ranging from 68° F. to 80° F. It was found that those oysters from the Sound, where the water temperature is much lower than in the Harbour, spawned in about half an hour at 68° F.–72° F., while the oysters from the warmer harbour waters required several hours' exposure to this temperature before spawning occurred, or, on the other hand, they could be induced to spawn in half an hour by increasing the temperature from 73° F. to 80° F.

During 1925 and 1926 the heaviest spawning of oysters in the harbour was found to occur after the water had reached and maintained for a few days a temperature of from 68° F. to 70° F. In both years the majority of the oysters spawned at the end of the July full moon tidal period, when the temperature of the water increased as the result of the greater range of tide during this period. Prytherch maintains that the effect of the moon on the spawning of the American oyster is only indirect, through the change it produces in the vertical and horizontal movement of the water over the oyster beds leading to an increase of temperature.

The discharge of spawn occurs near or at the time of high water. From 21st July to 29th July, 1926, the water temperature on the last of the ebb tide and at low water was often from 68° F. to 79° F., and yet the oysters failed to spawn. The same oysters, when placed in water pumped at high tide and warmed to the same degree, spawned in a very short time. It was observed by Prytherch, also, that oysters kept in floats always spawned near the time of high water, at temperatures ranging from 68° F. to 75° F., and never at low water, though it was several degrees warmer. In analysing the factors of temperature, salinity and hydrogen-ion concentration at the time when spawning occurred, it was found that the hydrogen-ion concentration or pH value of the water showed the greatest difference. In all the observations but one, the water had a pH value ranging from 7·8 to 8·2 when spawning occurred, while in the exceptional case the pH was 7·6 and the water temperature 73° F.

The failure of the oysters to spawn at low tide, when the temperature of the water is often above 68° F. is thought by Prytherch to be due to the low alkalinity of the water at this stage of the tide, as indicated by the pH readings.

Summarizing the studies of spawning in Milford Harbour, Prytherch concludes that the most important controlling factors are the temperature of the water, the range of the tide, and the hydrogen-ion concentration.

T. C. Nelson (1929) found that oysters may spawn even though their gonads may not be developed to their maximum extent; spawning may occur, in fact, when the gonad is but poorly developed. He considers, however, that the crops of spat resulting from such spawnings are very poor.

Galtsoff (1930*a*) found that the duration of the spawning period of *O. virginica* varied from 36 to 70 minutes, and the number of contractions varied from 56 to 135, the average number of contractions per minute of four oysters being 1·36. In the introduced Japanese species, *O. gigas*, he found that the duration of the spawning period varied from 15 to 59 minutes, and the number of contractions from 31 to 121, the average number of contractions per minute of four oysters being 1·96. The contractions of the two species are not strictly correlated, however, for they were not observed at the same time and under identically similar conditions, the record for *O. virginica* being obtained in July, 1929, and that for *O. gigas* in October of the same year.

With regard to the larviparous oyster of the Pacific coast of the United States (*O. lurida*), T. C. Nelson (1928) states that it spawns at the same temperature as *O. edulis*, viz., 59° F.–61° F.

In the oviparous Japanese common oyster (*O. gigas*), Amemiya (1928) observed numbers of oysters spawning both on the beds and in glass jars. On the beds the oysters spawned about 30 to 40 minutes after they were submerged (to a depth of about a foot) by the water on the flood tide. Unfortunately no temperatures or other physical conditions are stated, and it is not recorded whether the tide was spring or neap, though in another place Amemiya states that the ordinary temperature which prevails in central Japan at the spawning season is about 77° F. Amemiya observes that "Most probably in the natural oyster bed, the impetus of the fresh current of the flood tide causes the stimulus to spawning just as the change of water in the laboratory achieved the same result".

Amemiya discusses the ejection of spawn by *O. gigas* when the water recedes from the oysters on the ebb tide. He remarks that this snapping of the shells on the ebb tide may be observed at times other than the spawning season of the oyster, and he concludes that it is a "form of activity connected with some other habits of the oyster, and is not employed solely for the purpose of spawning". This snapping of the shells of oysters as the tide leaves them may be seen on all oyster beds at all times of the year; it causes the ejection of the accumulations of waste material rejected by the palps and carried along a ciliated track to the mantle edge. By this means the mantle cavity is rid of all accumulated waste prior to the shells being left exposed to the air, where they must remain without a water circulation till the next tide covers them. It is in no way connected with spawning.

Amemiya (1929) also states that *O. gigas* has one spawning season in a year, and at the end of the season the animal is entirely exhausted.

With regard to the Japanese larviparous species, *O. denselamellosa*, Seno (1929) states that, in the Seto Inland Sea, spawning occurs most actively from the end of June to the middle of July, when the water temperature ranges from 70° F. to 73° F.

In the case of the Bombay (oviparous) oyster (*O. cucullata*), Awati and Rai (1931) found that, along the coast of Bombay, the optimum temperature for spawning ranges from 80° F. to 87° F., and the optimum density is about 1·020.

On the coast of New South Wales spawning is a most irregular process. With the warming of the water during the spring, the gonad develops rapidly and may become fully developed by the middle of December. Spawning frequently occurs in such oysters during the abnormally high spring tides round about the Christmas period. These oysters frequently spawn again in late summer. If, however, owing to an abnormally cool spring the gonad develops slowly, spawning may occur in January, or even as late as April or May. In other cases there may be several partial spawnings throughout the summer months. Spawning is not confined to the summer months, however, for on rare occasions intermittent light spawnings may occur right throughout the winter, as shown by an irregular winter catch of spat. These have been noticed in the Hawkesbury River and at Port Stephens. I have seen, in November, young spat attached to the mangrove sticks from the size of about a millimetre ranging up to about 10 millimetres, which must have developed from winter spawn. When, as is usual, spawning is completed by the end of summer, the gonad passes through a quiescent period during the winter as far as the development of ova or sperms is concerned, but activity is displayed in the storage of glycogen in the vesicular tissue in preparation for the development of ova and sperms in the spring. The formation and storage of glycogen, however, does not appear to be so heavy as in the European oyster (*O. edulis*), which is frequently spoken of as fattening during the winter months. The size of the gonad of the New South Wales oyster in the winter is not comparable with its size in the summer, when it is distended with spawn. Nor is it appreciated to the same extent as food by the public, who prefer it with a well developed gonad. The winter in New South Wales is a period of greatest shell growth in preparation for the active development of the gonad during the spring and summer.

The irregularity of the spawning period in this species is no doubt largely accounted for by the fact that the great bulk of the oysters in New South Wales are grown in the tidal zone where temperature fluctuations, varying from cold water to hot sun in the course of a few hours, are enormous.

The vertical range of the New South Wales oyster extends from about mean high water level to a depth of upwards of 20 metres, and prior to the seventies of last century most of the oysters marketed were taken from permanently submerged beds. About 1870, however, the activity of a small worm (*Polydora ciliata*), which appears to have first made its appearance in the Hunter River, began to kill off vast numbers of these oysters. Gradually the worm spread to other rivers, and at the present day it is impossible to raise oysters on submerged beds in most of the rivers of New South Wales. The Manning and Bellinger Rivers are, however, notable exceptions; in the former the worm is present, but does very little damage, and in the latter it does not appear to occur at all. The mud worm has therefore forced cultivation to the tidal zone, where it can be combated.

If the oysters in New South Wales were grown on permanently submerged beds, where the temperature is not subject to the violent fluctuations characteristic of the tidal zone, it is reasonable to expect that spawning would be more regular in its occurrence, and an indication of an impending spawning given by the gradual rise in the temperature of the water, as obtains in America, where the bulk of the oysters are grown in situations never bared by the tide.

Experiments in Artificial Fertilization.

Experiments were carried out at various periods in order to study the embryonic development of the oyster, the rate of growth, and the influence of temperature on the growth-rate, and to compare the rate of development with that of other oysters, particularly *O. virginica* of the Atlantic coast of America.

At Port Macquarie in 1924 artificial fertilization experiments were carried out in water of four different densities. In A, ordinary estuarine water with a density of 1·021 was used; in B, tank water was added in sufficient quantity to lower the density to 1·015; in C, to 1·011; and in D, to 1·005. The initial temperature of the water in each case was 74° F. It was found that the rate of development decreased as the density was lowered. The free-swimming stage was reached in A in 8 hours; in B in 9 hours; and in C in 10¾ hours. No free-swimming embryos at all developed in D, and an examination 21 hours after the mixing of the eggs and sperms showed that fully 50% of the eggs had remained unfertilized, and of those in which fertilization had been effected, little development had taken place. There was no development beyond the morula stage.

The embryos in A, B, and C were kept under observation for 70 hours, and in each case appeared to develop quite normally. The temperature of the water varied during the course of the experiment from 70° F. to 74° F.

While the oysters were spawning on a bed at Port Macquarie on 5th April, 1924, a large accumulator jar was filled to a depth of 8 inches with water over the spawning bed. The temperature of the water was 70° F., and its density 1·021. In this water four spawning oysters were placed and allowed to remain till 10.30 p.m. on the same day. In a short time the bottom of the jar was white with spawn. At 9 p.m., seven hours after fertilization, a small percentage of the embryos was found to be swimming on or in the vicinity of the bottom. The temperature of the water had fallen in the meantime to 68° F. At 10 p.m., eight hours after fertilization, numbers of embryos were found at the surface of the water, having swum vertically a distance of eight inches. A quarter of an hour later numerous short, white columns were seen extending downwards from the surface of the water; these were formed by congregations of embryos swimming towards the bottom. These columns are characteristic of the free-swimming, embryonic stage of the oyster. The embryos are continually swimming from the bottom to the surface, where they may remain for some time attached to the surface film, before making their way again to the bottom. They rise singly, and in their passage cannot be detected by the naked eye, but in their journey downwards they follow each other in well-defined lines. The columns are distributed right throughout the water, but are usually more prevalent near the sides of the jar. Vibration of the containing vessel, which causes a slight agitation of the water, stimulates the embryos at the surface to descend. The columns are most numerous near the surface of the water, but nevertheless many of them extend right to the bottom (Pl. xxiv, fig. 31).

After about 9 hours the great bulk of the embryos were freely swimming in the jar, and the bottom was practically clear.

At 12, midnight, on the day following the spawning, 34 hours after fertilization, the first embryos were seen completely covered with shells, the hinge-line being straight. The temperature during this time had varied from 68° F. to 70° F. The

average measurements of 20 larvae at this time were: length, 67μ; depth, 53μ; and the length of the hinge, 45μ.

It is characteristic of the larvae to swim in circles, and when moving from the bottom to the surface of the water, their course is therefore a long spiral of narrow diameter. Shelled larvae, on reaching the surface, may remain there for some considerable time with the valves of the shells extended, the ciliated velum attached to the surface film. A sudden withdrawal of the velum and snapping together of the valves causes the larva to sink to the bottom, where, in a very short time, it will open its shell, protrude the velum, and again swim spirally to the surface. Larval life, then, consists of voluntary movements up and down in the water, the larvae at the same time being carried hither and thither by tides and currents.

After three or four days the larvae, which till then were practically devoid of colour, developed a bluish or bluish-grey tint, most pronounced dorsally in the vicinity of the hinge.

At the end of 5 days the average dimensions of the larvae were as follows: length, 75μ; depth, 58μ; and the length of the hinge, 50μ (Pl. xxv, fig. 32). No special effort was made to carry the larvae through the whole of their free-swimming period, and on the sixth day the prolific development of infusoria in the water rendered it necessary to discontinue the experiment.

On a number of occasions the rate at which the embryos swam was measured with the aid of an ocular micrometer. A drop of water containing embryos was placed on a microscopic slide and a cover-glass gently lowered over it. By means of a stop-watch the time taken by the embryos to traverse the full length of the micrometer scale was ascertained, and an average obtained from numerous counts. The total length of the micrometer scale was 1/31 inch. On one occasion, embryos 69 hours old, which had not developed shells, took an average of 2½ seconds to swim the length of the scale; at the rate, in other words, of approximately 46 inches per hour. On another occasion, embryos 24 hours old were timed to swim at an average rate of approximately 39 inches per hour, and at the end of 30 hours at about 58 inches per hour. The rate of swimming of different batches at different ages, therefore, ranged from 39 inches to 58 inches per hour. In view, however, of the artificial conditions under which these embryos were living, lacking efficient aeration, etc., it cannot be assumed that their progress under natural conditions would be the same; their vitality and therefore their activity would in all probability be greater. The rate given here may be taken as a conservative estimate of the progress of the embryos under natural conditions.

In order to determine the part played by the temperature on the rate of development of the embryos of *O. commercialis*, the following experiments were carried out.

Eggs and sperms were mixed in water obtained from the Hawkesbury River in two large accumulator jars, the depth of water in each case being 10 inches and the density 1·016. In one case, A, the initial temperature of the water was 77° F., and in the other, B, 64° F.

In A, at the end of 1½ hours many ova had completed their first division; after 1¾ hours numbers of ova showed a partial division into two micromeres and a deutomere; after two hours many showed four micromeres; after 2½ hours the early morula stage was seen; after 5 hours the embryos, now ovate in form, had reached the gastrula stage. After 6 hours several embryos had reached the

swimming (trochophore) stage, revolving in circles, the cilia beating so rapidly that, even with the critical definition of an oil lamp, they could not be distinguished. All embryos, when beginning to swim, revolve in small circles on the bottom, mostly, but not always, in a counter-clockwise direction. After 6¼ hours several embryos, when transferred from the accumulator jar to a watch-glass, were seen to leave the bottom and swim upwards for short distances. After 9 hours several embryos were found at the surface of the water in the accumulator jar, ten inches from the bottom.

During the course of this experiment, the temperature, which at the beginning was 77° F., varied from 76° F. to 80° F. The temperature was taken every half-hour and the mean determined as 78° F.

In experiment B, the temperature of the water when the eggs and sperms were mixed was 64° F., the depth of the water (10 inches) in the accumulator jar, and its density, 1·016, being the same as in experiment A.

At the end of an hour, some fertilized ova had become spherical and more opaque; the majority, however, were still more or less pear-shaped, though they had developed an opacity indicative of fertilization.

After 2 hours partial cleavages only were seen. After 2½ hours several embryos were seen with a division into two micromeres and a deutomere. After 3½ hours a number were seen with four micromeres; and after 5 hours the most pronounced development noted was an early morula. (It will be noted that this stage was reached in 2½ hours in experiment A.) After 11 hours many embryos had reached the early gastrula stage; at the end of 22 hours the first swimming embryos were seen revolving very slowly.

During this experiment the temperature varied between 55° F. and 65° F. The experiment was begun at 1.15 p.m., and from then till midnight the temperature fluctuated between 64° F. and 60° F. At 7 a.m. the following morning it had fallen to 55° F., whence it gradually increased till at 11.15 a.m. it had risen to 59° F. From midnight till 7 a.m. the following morning, no temperatures were noted; with the exception of this period the temperatures were taken half-hourly, the average working out at 62° F. Owing to a decided fall during the intervening blank period, the average for the whole period would be somewhat lower than this.

A comparison of the rate of development of the embryos in each experiment shows a marked disparity due to the difference in the temperatures. In the first case, at an estimated average temperature of 78° F., the free-swimming stage was reached in 6 hours, while in the second, at an estimated average temperature of 62° F., the time taken to reach the same stage of development was 22 hours.

An average temperature of 78° F. is somewhat higher than that which would obtain in the estuarine waters of New South Wales during the summer months, and therefore it may be presumed that the time taken by the oyster embryos to reach the free-swimming stage will be somewhat longer than 6 hours. The estimated average temperature which obtained during the course of the second experiment, 62° F., is considerably higher than that which prevails during the late winter months. During an investigation of a winter mortality of oysters in the George's River in 1924 (Roughley, 1926), I found that at a depth of 2 feet 6 inches below mean low water level the temperature varied, during July and August, from 50° F. to 59° F. The highest temperature reached was lower than the average of this experiment. During the winter, therefore, embryonic development will probably be somewhat slower than that indicated during the course of this

experiment. Of course, oyster embryos have rarely to face a winter temperature, but, as we have seen, winter spawnings do occasionally occur.

An effort was made both at Port Macquarie during 1924 and at the Hawkesbury River during 1925 to determine the duration of the free-swimming stage, following the method initiated by Stafford in Canada in 1907, which consisted of collecting the larvae in a plankton net daily, and making accurate measurements of the predominating size-group and recording the daily increase in growth.

During my six weeks' stay at Port Macquarie during the late summer of 1924, the plankton net was towed at least once a day, in various situations in the estuary, but on not one occasion were any oyster larvae found. After the spawning which occurred on the beds near the boatshed where my laboratory was situated, the net was used with greater frequency, at various states of the tide and in many and varied situations, both at the surface and close to the bottom in shallow water, but on no occasion were any larvae recovered. After the lapse of a fortnight the attempt was abandoned, but not before I felt justified in predicting that, in spite of the spawning, a set of spat was unlikely to occur. And so it turned out, though I did not receive any indication of the conditions which operated to destroy the larvae.

Again, at the Hawkesbury River during 1925, I used the plankton net at least once daily, not only near the State Fisheries boatshed at Brooklyn, which was fitted as a temporary laboratory, but in situations as far removed as Cowan Creek, near the entrance of the river into Broken Bay, and also many miles upstream. On every occasion oyster larvae were obtained, both in the straight-hinge and umbo stages, but never was there a definite preponderance of an age-group (Pl. xxv, fig. 33). This was rather surprising in view of the fact that examinations of oysters' gonads on many occasions previously had indicated that spawning occurred mostly during spring tides of either full or new moons. It became apparent that in the Hawkesbury River, during the period under review, the spawning was either not confined to spring tides, or the scattering of the larvae subjected them to a range of temperature sufficiently wide to cause a marked disparity in the growth-rate. Careful analysis of each day's plankton collection gave the impression that the spawning of the oysters in the Hawkesbury River during that period was occurring almost daily. The beds are distributed over a very great area, extending from the entrance to a distance of about 20 miles upstream, and also in numerous large creeks which bear heavy crops of oysters, extending in each case for many miles. Spread over such an area, a wide range of conditions is met with, both as regards salinity and temperature, and a uniform spawning period can scarcely be expected. On the contrary, one would anticipate great irregularity; and it is probable that under such conditions spawning occurs on different beds at different times throughout the summer months.

Most crops of very young spat which I have examined have shown a similar diversity of age, but on one or two occasions I have seen a general uniformity in the size of very young attached spat. In order to determine the length of the larval life of the oyster in New South Wales, therefore, one would have to be fortunate enough to carry out an investigation when the spawning and growth of the larvae showed a pronounced degree of uniformity.

In the absence of direct evidence of the length of larval life of the Australian oyster, an indication of its duration may be obtained by a comparison of the

growth-rate during the embryonic stages with that of the oyster of the Atlantic coast of America (*O. virginica*). At a temperature of about 80° F. the free-swimming stage in *O. virginica* is reached, according to T. C. Nelson (1921), in from four to five hours; and we have seen that, at a temperature of about 78° F., the same stage is reached in *O. commercialis* in six hours. Nelson also states (loc. cit.) that the larva is completely enveloped in shells within 24 to 36 hours after fertilization, while the Australian oyster took 34 hours to reach that stage at a temperature varying from 68° F. to 70° F. A difference in temperature of 2° as recorded in the American and our experiments would cause but a slight difference in the rate of growth, and it would appear, therefore, that the Australian oyster develops somewhat more slowly than the American oyster at the same temperature. If the subsequent growth of the larvae is maintained at the same relative rate, it is apparent that the free-swimming life of the Australian oyster will be somewhat longer than 14–16 days.

The absence of predominating size-groups in the Australian plankton and their more regular occurrence in the American plankton may be explained by the differences in the habitats of the parent oysters. In Australia the great bulk of the oysters occur in the tidal zone, where they are subject to great variations of temperature, leading to an irregularity of spawning; in America, however, most of the oysters are never exposed by the tide, often growing in water of considerable depth where there is a certain uniformity of temperature, which must tend to a greater uniformity of sexual development and a more regular spawning.

In the United States of America the determination of the occurrence of oyster larvae in the plankton is a question of considerable economic importance, as has been demonstrated by T. C. Nelson on a number of occasions. Oyster cultivation in America consists, in the main, of dumping large quantities of shells on the bottom to provide attachment surfaces for the larvae. Now, the efficiency of any form of cultch is dependent very largely on its cleanliness, and its cleanliness is governed by the length of time it remains in the water. Material which has been submerged for a month or two will not catch nearly so many oysters as that which has been in the water for a few days only, owing to the deposition of silt over the surface. If, then, an examination of the plankton reveals large quantities of oyster larvae of approximately the same age, it can at once be determined when the subsequent fall of spat may be expected, and the shells can be dumped on the bottom within a few days of the spatting. Thus the largest possible crop is obtained.

A knowledge of the length of larval life does not promise to be of similar importance to the oyster fisheries of Australia, at least so long as the bulk of the oysters are grown in the tidal zone, for spawning is always likely to be intermittent, and this naturally leads to an irregular catch of spat, extending over a considerable period.

Orton (1926), in *O. edulis*, determined that the larvae under natural conditions are retained in the mantle cavity of the parent for a period varying from 1 to 1½ weeks, and that, generally, settled spat may be seen in fair numbers in normally warm weather about a month after the first batch of white-sick oysters is seen.

Hori and Kusakabe (1927) succeeded in raising the larvae of the oviparous *O. gigas* to the setting stage in petrie dishes by feeding them on a minute alga

(*Chlorella pacifica*). Under these conditions the length of larval life was 23 days, at a temperature which fluctuated between 73° F. and 90° F., the average at 10 a.m. being 82° F. The density of the water was about 1·019–1·021. It is reasonable to presume, however, that this rate of development will be found to be considerably slower than that which obtains under natural conditions.

In the Japanese larviparous oyster (*O. denselamellosa*), Seno (1929) states that fixation takes place about 4 weeks after spawning, at an approximate water temperature of 68° F.

Further observations on the development of oysters of various species may be noted for comparison with that of *O. commercialis*.

In *O. edulis*, Huxley (1883) states that the larva at the time of ejection from the parent measures 1/150 inch (0·17 mm.) long. In the larviparous Australian oyster (*O. angasi*), I have found shelled larvae as large as 0·2 mm. The largest shelled larvae found by Stafford (1913) in the mantle cavity of the oyster of the Pacific coast of America (*O. lurida*) measured 0·186 mm.

In *O. edulis;* Spärck (1925) states that the larvae cannot complete their development in water with a salinity below 24–25‰. In the same species, Orton (1926) found that the larvae develop to the shelled stage in about a week at summer temperatures.

Orton (1927) also states that artificial fertilization cannot be performed at present in the case of *O. edulis* with much chance of obtaining a normal rate of development, for when embryos are taken away from the parent, and are left in unchanged ordinary sea water, development soon becomes abnormal.

In *O. virginica*, T. C. Nelson (1921) states that when first completely covered with shells the larvae measure 0·06 mm. in length. Prytherch (1923) found that at a temperature of 80°–85° F. the larvae invariably died; the most successful development occurred at a temperature of 70° F. T. C. Nelson (1921) had already noted that the embryos begin to swim in from 4 to 5 hours after fertilization at a temperature of 80° F.

In *O. gigas*, Hori (1926) found that the larva forms a bivalve shell in from 2 to 3 days; its dimensions at this stage are: length, 0·07 mm.; height, 0·06 mm.; and length of hinge, 0·04 mm. Seno, Hori and Kusakabe (1926) determined that the optimum temperature for larval development of *O. gigas* lies between 73° F. and 79° F., and the optimum density between 1·017 and 1·021. Amemiya (1928) states that the larval shells of *O. gigas* grow large enough to cover the whole body and velum in from 3 to 4 days. The time required for the development of *O. denselamellosa* is stated by Amemiya to be much longer than that of other Japanese oysters.

The Sex Ratio of O. commercialis.

During the past 15 years I have on many occasions examined microscopically the gonads of this species, and whenever a batch was worked through I have invariably noticed that the females predominated. In order to determine the ratio of females to males over as much of the State as possible at approximately the same time of the year, I had forwarded to me during January and February, 1927, through the courtesy of State Fisheries, 100 oysters from the most important oyster producing rivers in the State, numbering 27 in all. From three of these rivers two consignments were received, so that a total of 3,000 oysters was examined. The proportion of females to males is shown in the following table:

Locality.	♀	♂	Locality.	♀	♂
Tweed River	64	36	Brisbane Water	68	32
Brunswick River	62	38	Hawkesbury River	70	30
Richmond River	74	26	George's River	85	15
Evans River	82	18	Crookhaven River	78	22
Clarence River	81	19	Currumbene Creek	70	30
Sandon River	72	28	Clyde River	68	32
Bellinger River	66	34	Moruya River	78	22
Nambucca River	82	18	Wagonga River	68	32
Port Macquarie	82	18	Wallaga Lake	70	30
Manning River	75	25	Wapengo River	58	42
Wallis Lake	70	30	Nelson Lake	84	16
Lower Port Stephens	54	46	Merimbula Lake	64	36
Upper Port Stephens	84	16	Pambula River	76	24
Hunter River	88	12			

It will be noted that in every case the females predominated, ranging from 54% in Lower Port Stephens to 88% in the Hunter River. The average percentage was: females 73%; males 27%. In other words, there were 2·7 times as many females as males. The highest percentage of females I have ever found occurred in a batch of 100 large oysters from the Hawkesbury River, which were examined on 31st March, 1931. The females numbered 96%.

Toward the end of this investigation I opened 15 very young oysters which were attached to the larger ones received from Brisbane Water, and noticed that the gonads were just showing signs of development, the ducts being in most cases just visible. Every one was found to be a male, containing actively moving sperms, but no ova in any stage of development.

In a further batch received from Brisbane Water there were 8 very young oysters, and these, too, proved to be all males. In the consignment from the Clarence River, also, there were 9 very young oysters attached to the older ones; again, all were males. Amongst those received from Lower Port Stephens were 19 very young individuals, all of which were males. Similarly, 16 young oysters from the George's River, and 10 from the Manning River were all found to be males.

A sex-change during the course of development was at once indicated, and steps were immediately taken to procure for examination further samples of very young oysters. A hundred young oysters were received from each of the following localities: Port Macquarie, Lower Port Stephens, Pambula River and the Hawkesbury River.

The oysters from Port Macquarie were received on 1st February, 1927, and it was stated by the Inspector of Fisheries who forwarded them that they were from a crop that had set during March the previous year. This would give them a maximum age of 10 months. The gonads were very poorly developed, the digestive diverticula in the bulk of them not having yet been obscured. It was found that 84% were males and 16% females.

The oysters from Port Stephens had caught on mangrove sticks which had been laid out in January, 1926, and were reputed by the Inspector of Fisheries to have attached in the following month. They were examined at the end of February, 1927, and their greatest age could not, therefore, have been more than 13 months.

There was, however, no uniformity in the size or age of the oysters, several crops of various ages being indicated. It was found that 66% were males, 19% females, 13% contained neither ova nor sperms, and 2% were hermaphrodite, containing well developed ova and motile sperms. Some of the older oysters had well developed gonads, and amongst these the females occurred in greater numbers than amongst the smaller individuals, which were almost invariably males. In two instances, however, very young oysters showed developing ova.

The oysters received from the Pambula River were generally larger than those from Port Stephens, and the gonad was much better developed, some showing very pronounced development. They varied greatly in size, however, and although they were described by the Inspector of Fisheries as being a year old, there was clearly considerable variation in the ages of different individuals. It was found that 65% were males; 28% were females; and 3% were hermaphrodite, containing ova and sperms in various stages of development.

It will be seen that in three samples of oysters from different rivers, the oldest of which were reputed to be not older than 14 months, the males considerably outnumbered the females. With very few exceptions, the youngest oysters, in which the gonad was in an early stage of development, were males. It is quite apparent, therefore, that the Australian oyster (*O. commercialis*) undergoes a sex-change, but it is not yet quite clear whether it always functions first as a male. It certainly does in the great majority of instances, but a few individuals have been examined with the gonad in a very early stage of development, in which ova and not sperms were developing. It is possible, of course, that these oysters were not exceptions, that they had already functioned as males, though of this no indication could be obtained. It may be regarded as significant, however, that in the first instance, a total of 77 very young oysters received from five different rivers proved, without exception, to be males, and the examination of further batches during the following month showed a small percentage functioning as females. Is it possible that some of the oysters had spawned as males in the meantime, and had then begun to develop ova?

When this indication was given that a sex-change occurred, a careful examination was made of each individual in an effort to locate both ova and sperms in the one oyster. Previously, microscopical examination had been confined to the use of a 2/3 inch objective with ordinary illumination, which was quite serviceable for detecting ova or sperms when the field was confined to either one or the other. If, however, both ova and sperms occurred in the same field, there was a risk of overlooking the sperms, which are relatively very small, or, perhaps, confusing them with the Brownian movement of particles of protoplasm from damaged ova. For this reason, then, every smear from the gonad was subsequently examined first with a 2/3 inch objective illuminated through an ordinary condenser, and then with a 1/10 inch objective using a dark-ground condenser, with which sperms could be detected with unmistakable clearness. Later, on account of the time occupied in the use of the dark-ground condenser, the examination for sperms was made by dark-ground illumination using a 1/3 inch objective and an expanding stop, by means of which a sufficiently large and perfectly clear image of sperms was obtained. By this means a number of oysters was found with both ova and sperms in the gonad. Mention has already been made of the fact that an occasional young oyster contained both ova and sperms in the gonad, but it was found that this condition is not confined to the young stages, for, amongst 100 marketable oysters (approximately three years old) received from the Tweed

River, two contained both ova and sperms. In one both were in abundance, and in the other there were many sperms but relatively few ova. One individual was also received from the Hawkesbury River which contained both ova and sperms in abundance. A total of nine hermaphrodite oysters was found. It is possible that other examples of hermaphrodite oysters would have been found if both ova and sperms had been carefully searched for during the whole of the examination of the three thousand oysters previously referred to; it was unfortunately late in the examination that an indication of a sex-change was revealed, and therefore a small proportion only of the three thousand oysters was examined critically with this object in view.

Microscopic sections were prepared of three young oysters whose approximate age was twelve months, and of two large marketable oysters which I should estimate to be about three years old.

In an oyster taken from the Pambula River on 1st March, 1927, reputed to be about twelve months old, the shell of which measured $1\frac{1}{4}$ inches in length, most of the germinal ducts contained both ova and sperms (Pl. xxii, fig. 26, *o.*, *s.*), the sperms filling up the lumen of the duct, while the eggs were in various stages of development from the germinal epithelium. A few of the ducts contained eggs only, and in a few restricted areas the ducts were filled with well developed ova while spermatocytes were developing from the germinal epithelium. The follicles varied greatly in depth; in some situations where the vesicular connective tissue was narrow they extended as far as the digestive diverticula, in others, where the depth of the vesicular tissue was much greater the follicles extended only about half way. Most of the follicles were of small diameter; in most situations the wall of the follicle had attached to it eggs in various stages of development, and enclosed an abundance of ripe spermatozoa; in others the follicle enclosed large, well-developed eggs with spermatozoa in the spaces between them (Pl. xxii, fig. 27, *o.*, *o.*^{1}, *s.*).

In this oyster, therefore, sperm development appears for the most part to have preceded egg development, while in restricted areas both eggs and sperms continued to develop side by side.

In an oyster taken from the Hawkesbury River on 3rd February, 1927, which measured $1\frac{1}{2}$ inches long, the gonad was much better developed than that just described, and contained eggs and sperms in about equal proportions. For the most part developing eggs were attached to the walls of both the ducts and follicles, and surrounded masses of ripe spermatozoa. It is clear that the bulk of the sperms have developed prior to the development of the eggs, but in some situations developing eggs and spermatocyte adjoin each other in the same duct or follicle, in some cases with eggs, and in others with spermatocytes, predominating. Active egg and sperm development is therefore taking place concurrently.

In a twelve months old oyster taken from Port Stephens on 28th February, 1927, the shell of which measured $1\frac{1}{8}$ inches, the gonad was but poorly developed, the ducts just becoming apparent at the surface. In this oyster eggs were found to predominate, and many of them appeared to be fully developed. Many of the ducts were filled with eggs only, while eggs only were developing from the germinal epithelium; in others the bulk of the lumen was filled with eggs with sperms clustered in the spaces between them, sometimes with a dense mass lying against the ciliated epithelium. In a few of the ducts and follicles the development of both eggs and sperms was very active. From the advanced stage of egg development in this oyster, it is apparent that the proliferation of both eggs and sperms has gone on side by side for a considerable time.

In a marketable oyster taken from the Tweed River on 22nd February, 1927, in which the gonad was very well developed, microscopic examination of sections of the whole of the gonad showed that on the right side an extensive sperm development had been followed by very active egg development; in one or two of the ducts eggs predominated, but all contained spermatozoa in contact with the ciliated epithelium, while deeper down, the follicles, which were for the most part small and separated by considerable amounts of unabsorbed vesicular tissue, were all bordered by developing ova and enclosed compact masses of sperms (Pl. xxiii, fig. 28). In some situations both eggs and spermatocytes were actively developing in the same follicle. Dorsally, between the origins of the mantles, this condition gave way to a preponderating sperm development, whilst on the left side the gonad almost entirely consisted of testis; the ducts were filled with spermatozoa, and the follicles lined by spermatogonia and spermatocytes, which enclosed large masses of sperms. In a few isolated situations, however, a few eggs were found attached to the follicular wall, while deep down in the testicular tissue an occasional follicle was seen which contained ova only in various stages of development. It is apparent, therefore, that, although great numbers of spermatozoa were produced before a general tendency occurred to produce eggs, a few eggs were differentiated very early in the sexual development of the oyster during that season. If, when this oyster was alive, some of the sexual products of the gonad on the left side were examined, it is probable that sperms only would have been found, and the oyster would therefore have been identified as a male; if, however, the sexual products were obtained from the right side, an abundance of both eggs and sperms would have been found, and the true hermaphrodite condition of the gonad disclosed.

In another marketable oyster from the Tweed River which occurred in the same batch as that just described, the gonad was well developed, the areas of unabsorbed vesicular tissue being very small and inconspicuous. Great numbers of eggs and sperms occurred in about equal bulk throughout the whole of the gonad. The ducts, most of which had coalesced with their neighbours, contained for the most part an abundance of spermatozoa in contact with the ciliated epithelium; in some cases, however, eggs lay against the cilia; and in others again the eggs and sperms were intermingled indiscriminately. The convoluted follicles (Pl. xxiii, fig. 29) were lined by eggs in active development, all stages from the smallest to the mature egg being abundant; they enclosed irregular masses of sperms. Sperm development, however, had almost entirely ceased, for very few spermatocytes could be found.

Observations on the sex-ratio of other species of oysters have been made by a number of authors.

In the European larviparous oyster, Dantan (1913) found that amongst one-year old oysters 76% were males, and 6% females; when two years old 84% were males, and 15% females; and when three years old 81% were males, and 18% females. Dantan also found that the largest oysters always contained spermatozoa, and that eggs are only found in those of medium size. He thought it likely that the placing of individuals under less favourable conditions favoured the formation of male elements.

Spärck (1925) found that in the years since 1919 there were at no time in the Limfjord more than 8% to 10% of females; while from 75% to 80% of individuals were found containing spermatozoa. In the colder waters of the Limfjord females are found principally amongst the older oysters.

Orton and Awati (1926) found that females predominated in the oviparous Portuguese oyster, *O.* (*Gryphaea*) *angulata*, which had been relaid on English oyster beds, the proportion varying from 60% to 88%.

Stafford (1913) states that the sexes of the American oyster (*O. virginica*) are approximately equal in numbers. Similar results were obtained by T. C. Nelson and other American authors.

In the Japanese oviparous oyster (*O. gigas*), Amemiya (1928) found that the sex-ratio varied with the amount of nourishment obtained. On a good fattening ground the ratio of females to males was 100 : 95 amongst the one-winter group, and 100 : 73 amongst the two-winter group. On a poor fattening ground, the ratio of females to males was 100 : 116 amongst the one-winter group, and 100 : 155 amongst the two-winter group. Amemiya concludes, therefore, that an abundance of food tends towards the development of a preponderance of females, and a paucity of food tends towards the development of males, thus to some extent bearing out Dantan's conclusion in the case of *O. edulis*.

In *O. mordax*, an oyster which occurs prolifically on the Great Barrier Reef and North Queensland coast, an examination of 100 oysters from Ethel Rocks, near Gladstone, on 9th March, 1931, yielded more than twice as many males as females, the percentages being: females, 29%; males, 65%; indeterminate sex, 6%. In a communication to me from the Marine Biological Station, Low Island, North Queensland, a little later, F. W. Moorhouse stated, however, that an examination of oysters of this species made by him at Low Island yielded 62% females; and 37% females; with 1% of undetermined sex.

A brief outline of our knowledge of the sex-change in various species of oysters has been given in the introduction. Following the more detailed discussion of the sex-change in the Australian *O. commercialis*, further observations on its occurrence in other species will serve as an interesting comparison.

Davaine (1852) and Hoek (1883) stated that a sex-change occurs in *O. edulis*, and they based their conclusions on the occurrence of developing spermatozoa in individuals which had recently functioned as females. It has long been established that spermatozoa develop in oysters which still retain embryos or larvae in the mantle cavity, and which, therefore, had recently spawned as females, but it remained for Spärck (1925) to produce direct evidence of the change from male to female. By boring through the shells of male oysters, and re-boring them later, it was found that the spermatozoa had given place to eggs. Spärck is further of the opinion that external conditions play a big part in regulating the sex, and an alteration in the surrounding conditions results in an alteration of the kind of sex.

In the Portuguese oyster, *O.* (*Gryphaea*) *angulata*, Amemiya (1925–6) found that after 75 individuals had been confined to a tank for a month, two had developed a hermaphrodite condition. He is of the opinion that in the oyster the genetic sex-determining mechanism, even though it may incline the future differentiation of the individual towards either the male or the female type of sexual organization, is not all-powerful; the sex of an individual is not irrevocably determined by this mechanism. In the two cases of hermaphroditism referred to above, the earlier phases of their gonadic differentiation were pursued under conditions of ample nutrition: such of the gonadic tissues as differentiated at this time therefore became ovarian tissues. When placed in the tank, however, where food was relatively scanty, the gonadic tissues which developed under these adverse conditions assumed the organization of testis. In other words, an abund-

ance of food leads to the development of the physiologically more expensive ovarian tissue, and a scarcity will tend towards the development of testicular tissue.

Amemiya (1929) examined the gonads of one-winter individuals of *O. gigas* by boring through the shells, and repeated the operation when they had passed the second winter. Two batches were examined. In the first batch, examined in 1928 (one-winter oysters), 119 were females and 58 were males. In 1929, the original 119 females were found to consist of 82 females and 20 males; and of the 58 males, 32 were found to be females, and 18 were males. In the second batch examined in 1928 (one-winter oysters), 209 were females and 145 were males. In 1929, the original 209 females consisted of 85 females and 34 males; and of the 145 males, 48 were females, and 39 were males. In the first batch 29% had changed sex, and in the second, 23%. The sex-change amongst the total oysters examined was 25%.

Amemiya considers it probable that at the beginning of every new spawning season, the sex differentiates independently of the sex of the preceding season, and that protandry does not occur.

In the Bombay oyster (*O. cucullata*), Awati and Rai (1931) found that amongst normally healthy individuals 56% were females, and 41% were males, 3% being hermaphrodite, while amongst those which were harbouring pea-crabs (*Pinnotheres*) in the mantle cavity, only 10% were females, and 83% were males, 7% being hermaphrodite. It is thought that the pea-crab influences the change of sex either by reducing the food supply of the oyster, or by bringing about a change in its general metabolism.

In view of the observations of the above-mentioned authors on the bearing which the abundance or paucity of food has on the sex-determination, I obtained several batches of oysters from various localities in an endeavour to discover whether the food supply has any influence on sex differentiation in *O. commercialis*.

On 17th March, 1931, I examined two batches of oysters from the Clyde River, one from a bed about 1½ miles from the entrance, described by the Inspector of Fisheries as the finest growing and fattening ground in the river; the other from outside the bar entrance, where the salinity of the water is always high, and growth is very slow. In the first batch the females numbered 66%; the males 33%; and one was hermaphrodite; in the second batch the females were 65%, and the males 35%. It may be presumed that food is much more abundant on the bed where growth is very rapid than in the locality where growth is very slow, yet the percentage of females was practically the same.

Again, on 25th March, 1931, I examined a batch of oysters from Burraneer Point, Port Hacking, where conditions are very unfavourable to oyster life. These oysters, although in most cases of considerable age, were small and stunted, with very hard shells. They were obviously growing where food is never abundant, yet 65% of them were females, and 35% males.

I have a further record of the sex of 100 oysters from Middle Harbour, Port Jackson, examined in 1922. Here, again, conditions are very much against rapid development, and the oysters rarely reach a marketable size, yet 64% of these were females, and 36% males.

Here are records, then, of three batches of oysters from areas where the conditions are very adverse for oyster development, and presumably where the food supply is poor, and in each instance the females greatly outnumbered the males. If an abundance of food tends towards the development of femaleness in

this species, a preponderance of males would certainly be expected. On the evidence, therefore, it does not appear that the food supply has an influence on the sex-determination of *O. commercialis*.

Sizes and Characteristics of Oyster Larvae at Various Stages of Growth.

During the course of the investigation on the Hawkesbury River at various times from January to May, 1925, copious measurements were made of the oyster larvae taken in the plankton net. The net was 12 inches in diameter and the usual conical shape; the material used consisted of bolting silk, No. 16, containing 157 meshes to the inch, which was the finest obtainable in Sydney. Unfortunately the mesh was not sufficiently fine to retain the early straight-hinge stages, so that there remains a gap between the largest of those raised by means of artificial fertilization and the smallest of those retained by the net. We have seen that the dimensions of the largest larvae developed by artificial fertilization were: Length, 75μ; depth, 58μ; hinge, 50μ. The smallest larvae obtained in the plankton net measured 145μ long; 133μ wide; and hinge 75μ. From this size to that of the fully grown larva a complete gradation was obtained (Pl. xxvi, fig. 34, A–G). Many hundreds of measurements were made by means of an ocular micrometer, using a 2/3 inch objective. It was found that larvae of the same length exhibited minor variations in depth, hinge-line or umbo, much of which may be attributed to their lack of symmetry, which makes uniform orientation difficult. The differences, however, were very slight, and a sufficient number of individuals was examined to allow of a fairly accurate average being obtained.

The measurements given in the following table range, by single micrometer units, from 18 units (145μ) to 40 units (333μ).

Dimensions (in micra) of the Larvae of O. commercialis.

Length.	Total Depth including Umbo.	Hinge.	Umbo.
75	58	50	
145	133	75	
158	142	75	
166	145	83	
175	159		8
182	166		16
192	175		25
200	182		25
208	192		25
216	200		25–33
226	208		33
233	216		33
242	226		33–42
250	233		42
258	242		50
266	250		58
275	258		58
283	275		58
292	283		58
300	300		58
308	312–325		58–66
312	342–350		58–66
325	350–358		58–66
333	366		58–66

A series of measurements of the larvae of the American oyster (*O. virginica*) has been given by Stafford (1913); of the Japanese oviparous oyster (*O. gigas*) by Hori and Kusakabe (1926); and of the Japanese larviparous oyster (*O. denselamellosa*) by Seno (1929). For purposes of comparison they are shown in the following tables.

Dimensions of O. virginica, *in micra.*[1]

Length.	Depth.	Hinge.
69	55	48
76	62	48
83	69	48
89	76	48
96	83	48
103	89	48
110	96	48
117	96	55
124	103	55
131	110	48
138	110	55
145	117	55
152	124	55
159	131	55
165	145	55
172	148	55
179	152	55
207	193	
241	221	
276	262	
310	290	
345	296	
379	345	
386	359	

Dimensions of O. gigas, *in micra.*

Length.	Depth.	Length.	Depth.
80	68	165	185
90	80	180	200
95	94	200	220
100	107	220	240
110	120	250	265
120	132	270	280
125	140	280	290
143	160		

Dimensions of O. denselamellosa, *in micra.*

Length.	Depth.	Length.	Depth.
148	126	325	370
235	198	347	370
280	251	372	382
295	251	403	387
325	248		

A comparison of the length-depth ratio of the larvae of the four species shows certain interesting differences. In the case, first, of *O. commercialis*, we find that, up to 300μ, the larvae are longer than deep; at 300μ, the length and depth are equal; from about 300μ until they set as spat, their depth is greater than their length.

In *O. virginica*, it is seen that in all stages of development, from the youngest to the fully-grown larvae, the length is greater than the depth.

A further striking contrast is seen in *O. gigas*, in which only the very youngest stages, i.e., those under 100μ long, are longer than their depth; from the 100μ stage till they set as spat, when they are 280μ long, their depth is greater than their length.

The measurements of *O. denselamellosa*, as given by Seno, show a variation so great, compared with the very small variations in the other species, that one is tempted to conjecture that either more than one species of *Ostrea*, or, perhaps, some other bivalve larvae, have been included in the measurements; or possibly that the

[1] Stafford's measurements are given in ocular micrometer units, each of which in terms of the stage micrometer is 6·9. His units, therefore, multiplied by 6·9, give their value in micra.

orientation of the larvae has been unsatisfactory. Up to a length of 295μ the length remains constantly greater than the depth, but of the two larvae recorded with a similar length of 325μ, one has a height of 248μ, and the other a height of 370μ. A difference in the height of 122μ in two individuals of the same species in which the length is the same is an extraordinary variation, anything approaching which does not appear ever to occur in *O. virginica*, and certainly does not in *O. commercialis*. Stafford records a number of variations from his average measurements, and the greatest of them is a difference in the height of 27μ in a larva whose length was 138μ. The bulk of the variations, however, rarely exceed 6μ. In my own measurements, it was exceptional to find a variation greater than 8μ; the greatest variation was a difference in the height of 20μ in a larva which measured 258μ in length.

Summarizing the dimensions of the larvae of *O. commercialis*, *O. virginica*, and *O. gigas*, it may be said that *O. commercialis* is longer than deep in all but the latest stages of larval growth; *O. virginica* is always longer than deep; and *O. gigas* is deeper than long in all but the very earliest stages of larval life.

Unfortunately, progressive measurements of *O. edulis* do not appear to have been recorded; they should prove highly interesting for comparison. Boury (1930) states, however, that they measure between 200μ and 230μ long when they pass from the straight-hinge stage to the umbo stage. The proportions of a larva at the beginning of the free-swimming stage are: length, 100; height, 90; hinge, 45. Larvae in the straight-hinge stage varied from a length of 165μ and a height of 152μ, to a length of 229μ and a height of 203μ. In the umbo stage they varied from a length of 200μ and a height of 183μ, to a length of 275μ and a height of 260μ. The larvae of *O. edulis*, therefore, resemble those of *O. virginica* in the respect that their length is always greater than their height.

We are now in a position to compare the length of fully-grown larvae of the Australian oyster with those of other species. Marked differences obtain, as the following tabulated list shows:

O. edulis (larviparous) 270μ	*O. virginica* (oviparous) 386μ
O. lurida (larviparous) 255μ	*O. gigas* (oviparous) 280μ
O. denselamellosa (larviparous) .. 380μ	*O. commercialis* (oviparous) .. 330μ

Measurements of the thickness of larvae are not easy to obtain on account of the difficulty of standing them on their edges. By manipulation with needles, however, I succeeded on several occasions in leaning the larvae in an almost vertical plane against other objects in the plankton, and obtaining measurements. They were found to possess very considerable thickness, much greater than was indicated when they were lying flat (Pl. xxvi, fig. 34, H). For instance, one individual 230μ long was 175μ thick; another, 300μ long was 208μ thick; and a third 312μ long was 216μ thick.

Distribution of Larvae.

The early stages of larval oysters are captured in abundance when towing the plankton net at the surface of the water; here, however, the catches of older larvae decrease in proportion to their size. Fully developed larvae and those approaching full development were found to be more abundant near the bottom.

Oyster larvae, along with other matter suspended in the water, tend to collect in eddies, and I have repeatedly found that the best crops of oysters occur in situations where the currents, by the contour of the land, form eddies. Striking confirmation of this was obtained one afternoon when working on the Hawkesbury

River. During the morning a considerable quantity of fruit had been dumped overboard from a lighter, and in the afternoon was found to have collected in a small eddy opposite the boatshed in which I was working. I towed the plankton net for 10 minutes over an oyster bed some distance from the eddy, and, after washing the contents of the net into a beaker, I returned and towed the net in the area where the fruit was floating. The number of oyster larvae obtained in the second haul was very much greater than that secured in the previous one.

A study of currents and of the eddies formed by bays and headlands is of very great value in determining the location of good spatting grounds. Two of the most prolific spatting grounds in New South Wales are situated in Salamander Bay, Port Stephens, and in the bay east of the Hawkesbury River railway station. In the former, the water eddies strongly on the ebb tide, and in the latter on both flood and ebb tides.

In New South Wales the best spatting grounds are found in the estuaries near the entrances of the rivers. Here, the salinity of the water is consistently high, and the water contains much less sediment in suspension; consequently, material in the water which offers a surface for the attachment of spat remains comparatively clean. Oyster larvae cannot attach to a muddy or slimy surface. In such situations, however, the growth of spat is much slower than it is upstream, where the salinity is lower on account of the fresh water continually being received from the head of the river. As an instance of the effect of a higher salinity on the growth of oysters, an excellent example may be cited in the Hawkesbury River. In Cowan Creek, a tributary of the Hawkesbury River near its entrance into Broken Bay, the oysters, although occurring in most prolific crops, remain very stunted and rarely grow to a marketable size, even after many years. West of the railway crossing, however, where the effect of the fresh water from the source of the river and its numerous tributaries is continually felt on the ebb tide, growth is very rapid. If the stunted oysters from Cowan Creek are transferred to situations of lower salinity upstream they usually thrive and grow rapidly, provided that they are reasonably young when removed. The old, thick-shelled oysters from Cowan Creek rarely increase their size to any extent when transferred to other beds.

Another striking example of the effect of a high salinity is to be found in Port Hacking, situated a little south of Port Jackson. The amount of fresh water which enters Port Hacking is negligible, and the oysters, although very abundant, rarely grow to marketable size. If, however, the younger individuals are transplanted in the George's River, situated a few miles north, they quickly add to their shell growth, and in the course of eighteen months or two years develop into good marketable oysters.

An oyster larva begins to feed when it becomes a trochophore. It is then a naked cluster of cells, and swims by means of cilia strongly developed on the anterior surface. When the larva is first enclosed within shells the hinge-line is straight or slightly concave. From then till the hinge is obscured by the umbos the larva is usually referred to as being in the straight-hinge stage. At first the larva is pale grey in colour and semi-transparent; it gradually develops a bluish tint towards the dorsal margin. It is now provided with an alimentary canal in one loop, consisting of a primitive mouth leading through a short oesophagus into the stomach, which is merely a dilation of the alimentary tract, and this constricts posteriorly to form the short intestine, ending in the anus. The digestive diverti-

cula shortly become apparent as yellowish or yellowish-brown cells partially surrounding the stomach dorsally. The ciliated end of the larva (anterior) has now developed into a contractile velum, the cilia of which are longer and stronger than in the embryo. The velum is withdrawn inside the shell when it is closed and expands and projects beyond it when the shell opens to allow the larva to swim.

In *O. commercialis* the straight-hinge stage persists till the larva reaches a length of about 175μ. From thence onwards the hinge begins to become obscured by the development of the umbos. In *O. virginica* the umbos begin to show prominently when the larva is 172μ long (Stafford, 1913), and in *O. gigas* when it is 95μ long (Hori and Kusakabe, 1926). Coincidental with the development of the umbos, another organ of locomotion, the foot, begins to show prominently within the shell. For some time after the foot becomes visible it does not appear to be protruded beyond the edge, but can frequently be seen to move within the shell. It is situated behind the velum and oesophagus and anterior to the posterior adductor muscle; ventrally it extends in an antero-posterior direction along the line of the thickened edge of the mantle. With the development of the umbos the opacity increases, due partly to the increased thickness of the shells and the enclosed tissues, and partly to the deposition of pigment. The adductor muscles, of which in the larva there are two, an anterior and a posterior, can now be distinguished, and the digestive diverticula have developed a brownish hue.

The smallest stage at which I have seen the foot protruded beyond the shell is when the shell is 230μ long (240μ in *O. virginica*—Stafford). From thence till the larva settles down as spat the foot is used to enable it to crawl about.

When the larva is fully grown, and is ready to attach to some object as a spat, its opacity has increased very greatly and it is heavily pigmented. The shell, viewed by reflected light, is bluish-brown in colour and of a horny consistency; it shows concentric lines of growth, the spaces between them becoming wider towards the growing edge; they vary in number from 9 to 12. When viewed by transmitted light, the general colour is a yellowish-brown. Closer colour analysis shows that the dorsal region, in the vicinity of the hinge and umbos where the digestive diverticula are situated, is very dark brown, the middle area of the larva is yellowish-brown, sometimes with a greenish tinge, and the ventral edge pinkish-brown. The adductor muscles are bluish-grey, and the anterior prolongation of the foot is pink.

The mouth (Pl. xxvi, fig. 35, *mo.*) is situated between the velum and the foot; it leads by way of the ciliated oesophagus (*œ*) into the stomach (*s*), which lies below the hinge and is elongated antero-posteriorly. The intestine (*i*) leaves the stomach near its posterior border on the left side, immediately coils upwards and extends anteriorly into the left umbo (*l.u.*), coiling downwards when near the edge of the shell, and then extending backwards with a deep ventral loop, terminating in the anus (*a*) situated above the posterior adductor muscle. The epithelium of the whole of the digestive tract is ciliated, that of the stomach more strongly than the rest. The thrust of the cilia causes the food to revolve in a counter-clockwise direction. The dark brown cells of the digestive diverticula (*d.d.*) now completely envelop the stomach, and extend dorsally into both right (*r.u.*) and left (*l.u.*) umbos. The adductor muscles are oval in shape, the long diameter extending vertically; the posterior adductor (*p.m.*) is somewhat larger than the anterior (*a.m.*).

The velum (*v.*) occupies practically the whole of the area between the anterior adductor muscle and the median vertical line, i.e., anterior to the oesophagus. The

cilia are extremely long and powerful, and can be plainly seen through the shell when it is folded at rest. When protruded and extended it opens up to form a large, oval pad, exceeding the greatest width of the opened shells when seen swimming towards the observer's eye (Pl. xxvi, fig. 34, I). The foot, when at rest, occupies the space between the posterior adductor muscle posteriorly and the oesophagus anteriorly, extending ventrally to the edge of the mantle (Pl. xxvi, fig. 35, *f.*). It exhibits a pronounced prominence posteriorly, resembling somewhat the heel of the human foot, and the anterior prolongation extends forward in close proximity to the edge of the mantle, as far as the mouth. The velum is protruded much more frequently than the foot, and usually precedes the protrusion of that organ. Prior to the protrusion of the foot, it exhibits considerable movement within the shell, and the tip may be frequently projected and withdrawn. It gives the impression of great nervousness. When the whole of the foot is protruded beyond the shell (Pl. xxvi, fig. 34, M), it stretches and becomes narrower as it extends; the tip fastens to the bottom and a sudden contraction draws the shell after it. By a continued repetition of this movement the larva is capable of crawling considerable distances. When fully extended the length of the foot is about equal to the length of the shell.

On several occasions it was noticed that the foot enabled the larva to creep over the surface of the watch-glass in which it was being observed, by the contact of the cilia on the base of the foot with the bottom, the movement of the larva in this case being a slow and even progression forwards, as distinct from the rather violent jerks which ensue from the contraction of the foot when the tip is attached to the glass.

The foot is ciliated over the whole of the protrusible surface, the cilia along the ventral surface being larger and stronger than those elsewhere. There is a median groove on the ventral surface (L). The upper half of the portion protruded beyond the shell is pale magenta in colour; the lower half is pale yellowish-brown.

When the valves of the shells open the right and left mantle-folds extend to their edges, and are withdrawn a short distance within the shell when they close, the thickened edge of the mantle (Pl. xxvi, fig. 35, *mn*) showing as a well defined line extending from the anterior edge of the anterior adductor muscle to the posterior edge of the posterior adductor.

Conspicuous in the advanced stages of larval life are two very dark, almost black, pigment spots (otocysts, *o*), one on each side about the centre of the larva.

Toward the end of larval life the gills (*g*) begin to make their appearance, and may be distinguished in the posterior half of the larva as a transversely ridged band extending from behind the pigment spot downwards and backwards, terminating below the posterior adductor muscle. They are broadest anteriorly, and taper uniformly towards the posterior extremity.

When the velum and foot, or velum alone, are protruded the organs situated above them are pulled downwards an appreciable distance, the stomach then occupying the central region of the body.

The younger larvae are continually on the move from the bottom to the surface of the water, but as they become older they come to the upper layers with less frequency. Currents, however, carry them backwards and forwards and it is impossible to say where the progeny of any oysters will settle down as spat. Having completed its cycle as a free-swimming organism, the larva must

come in contact with some clean, firm surface, or within a short time, stated by T. C. Nelson (1921) to be about two days, it will usually die. Wells (1926) has recorded that when raising oyster larvae to the setting stage by means of a milk separator, a few were found which developed into perfectly healthy oysters without attachment, although there was plenty of material for them to attach to. The power of attachment appeared to be weakened or lost.

It appears to be characteristic of oysters everywhere that far greater numbers attach to the under surfaces of objects in the water than to the upper. This obtains in New South Wales, and care is always taken when laying out cultch to see that it is not placed vertically. Stone slabs may be leaned one against the other like an inverted V, or they may be stuck into the mud at an angle of about 45°. When mangrove sticks are used they may be bunched at an angle or laid horizontally. A reason for this peculiarity of the disposal of the young spat may be found in the fact that the upper surfaces of submerged objects quickly become coated with sediment, and that the heat of the sun's rays proves too severe for many of those spat that do attach to the upper surfaces of objects in the tidal zone. There is abundant evidence that the heat of the sun on a hot summer's day does occasionally kill considerable quantities of oysters in New South Wales, and it is only reasonable to expect that the newly attached spat, which, up to the time when they attach themselves, have spent the whole of their existence in the water where extremes of temperature such as are met with on the foreshores are not encountered, will be particularly susceptible to undue heat. Mangrove sticks, when used for the purpose of catching spat, are always bunched closely together, and are later (usually in the spring following attachment) transferred to beds where growth is more rapid; on these beds the sticks are stuck into the mud in an upright position a foot or two apart. Now, the greatest crops are always obtained on the inner layers of sticks, which are more or less sheltered from the sun's rays by the outermost sticks. Experience has shown that if these sticks are separated during the hot summer months, exposure to the sudden heat frequently proves fatal to many of the oysters, which have had no opportunity of acclimatizing themselves to such a temperature. For this reason, an effort is always made to transfer the sticks before the hot weather sets in.

It is at first sight rather surprising that more larvae should attach to the lower surface than to the upper, in view of the support which the upper surface provides, as against the necessity of clinging firmly and securely to the lower surface. It is not improbable, I think, that far greater numbers of fully grown larvae do alight on the upper surface than actually attach to it, and that they then crawl about in an endeavour to get beneath the object they alight on. If the surface is of too great an area, they can still swim in search of a more favourable object. In other words, I am firmly of the opinion that the larvae to some extent at least select the location of their attachment. Frequently, when I have laid out oyster shells to catch a set of spat, I have found that the majority were located in depressions, such as the indentations between the teeth along the inner margins of the shells. In such situations a certain amount of protection against abrasion is afforded when the shell is moved by tides and currents.

Then, too, there is a considerable discrimination exercised in the choice of material to which attachment is effected. The nature of the surface plays an important part in regulating the attachment. For instance, if the sticks of the Black Mangrove (*Aegiceras majus*), the White Honeysuckle (*Banksia integri-*

folia) and the Swamp Oak (*Casuarina glauca*) are laid out on the same bed, by far the greatest numbers of spat will attach to the Mangrove, which has a smooth, firm bark, while those of the Honeysuckle and Oak are rougher and more broken.

Still another factor appears to play a part in influencing the larva to seek the lower surface of an object for attachment. When nearing the completion of its development it exhibits a negative phototaxis. At this stage there appears on each side near the centre of each half of the mantle a dark pigment spot known as the pallial eye. "This spot", states Nelson (1925), "although unable to form an image like a true eye, is sensitive to light. Experiment shows that larvae which possess the pallial eye are sensitive to light, whereas those that do not are insensitive to normal illumination. In the presence of light the 'eyed' larvae of the oyster are stimulated and continue moving until they get into a shaded place, when they become quiescent. Since the pallial eye develops within 24 hours of the time of attachment, it follows that setting during the daylight hours is profoundly influenced by light. Since the larvae at the setting stage are active while exposed to light, it follows that during the day they would tend to collect in shaded places such as the under sides of shells, and such has been found to be the case."

The method of fixation has been actually observed by T. C. Nelson. It had long been maintained that the substance which cemented the larval shell to the object of attachment was secreted by the edge of the mantle. Stafford (1913), however, questioned the validity of this opinion, mainly because the mantle edge was engaged in the secretion of material for shell growth, and because of the "insurmountable difficulty in the bringing of the mantle margins far enough outside of the shell to be applied to the proper spot". Examination of sectioned larvae disclosed a byssus gland, capable of secreting matter that might be used in fixation, situated in the heel of the foot, which is capable of bringing the mouth of the gland to the necessary area on the outside of the shell. This byssus gland was found by Stafford to occupy, in fully grown larvae, a considerable portion of the inside of the foot; it consists of a median and two lateral lobes, with a main duct running through the median portion and continuing to the external opening at the end of the heel. In the youngest spat, on the other hand, the byssus gland is relatively inconspicuous, and its cells shrunken and collapsed. Clearly, at the time of fixation it has functioned very actively. Stafford further reasoned that, when secreting the cementing substance, the foot is thrust forwards and upwards until the point of the heel, on which the duct of the byssus gland opens, comes to the area of contact, and then the secretion is poured out, flowing between the shell and the substratum, until fully discharged, when the foot is withdrawn.

Nelson (1924) confirmed Stafford's contention that the cementing material is secreted by the foot, for he was able to watch the whole process in larvae that were crawling about on a piece of glass. He found, however, that the use of the foot differed somewhat from Stafford's conjecture. With the foot half extended and pointing directly away from the shell, its distal end flattened in contact with the glass, the left valve was held inclined to the glass at an angle of about 30 degrees. In this position the ventral edge of the shell almost touched the substratum. The ventral border of the mantle was extended until it came in contact with the glass where it remained for two minutes, and was then withdrawn. The foot was then slowly drawn in. "The extrusion of the mantle for a short period", Nelson states, "evidently aids in the quick and economical distribution of the cementing fluid as it is poured out of the byssus gland at the ventral edge of the left valve. The secretion hardens in less than 10 minutes."

The angle of attachment of *O. commercialis* corresponds with Nelson's estimate of the angle in *O. virginica*. A number of measurements with a goniometer eyepiece showed that the median line of the spat, i.e., the line of apposition of the right and left valves, lies at an angle varying from 27 to 32 degrees, while the angle of the upper surface of the right valve lies at an angle of about 56 degrees (Pl. xxvi, fig. 34, J). The average depth of newly attached spat of *O. commercialis*, i.e., the distance between the furthest edges of the right and left valves, is 216μ.

A note on the distribution of the larvae of *O. edulis* is given by Boury (1930), who states that during the day the larvae are found very near the surface when the water is calm, but that they sink to the bottom when the water is slightly agitated. At all stages of development the distribution remains the same.

A large amount of investigation has been carried out by American workers on the distribution of the larvae of *O. virginica* at various stages of their growth. T. C. Nelson (1925), for instance, found that in Barnegat Bay, New Jersey, the larvae rise during the flood tide and sink close to the bottom during the ebb. During the flood tide the heavier water of relatively high density creeps along the bottom while the lighter water of lower density remains above. In the zone of transition from the water of higher density to that of lower density, lying on the surface of the layer of dense water, the larvae of the oyster are found in relatively enormous numbers. By lowering or raising the infake of the hose used to pump the water through a plankton net a distance of a few centimetres, the oyster larvae may increase from a few dozen to thousands per hundred litres of water. By the beginning of the ebb tide or soon afterwards the two layers become mixed from top to bottom, when the larvae are found in greatest abundance close to the bottom, or actually on the bottom itself.

Prytherch (1928) found that in Milford Harbour, Connecticut, oyster larvae are most abundant at the time of low slack water, and when the flood tide develops a velocity of 0·6 foot per second, practically all the larvae sink to the bottom.

The majority of oyster larvae produced by the spawning beds in Milford Harbour are found to remain and set within 300 yards of its centre. This is accounted for by the oyster larvae remaining on the bottom during the greater part of the larval period and limiting their swimming activities to the time of slack water.

Setting or attachment of larvae was found to take place during low slack water and continued until the flood tide had developed a velocity of 0·33 foot per second, or 20 feet per minute.

Prytherch states that oyster larvae are not widely distributed by tides and currents.

A much wider distribution by tides and currents appears to obtain with the larvae of the Australian oyster, and their concentration does not appear to be confined to the close proximity of the spawning beds, but rather do the larvae tend to become widely distributed, and, as has been stated previously, to concentrate in eddies, which may be far removed from the region of the parent oysters. Illustrations of this may be seen in the prolific spat-catching grounds east of the railway station in the Hawkesbury River, and in Salamander Bay, Port Stephens. Before the introduction of mangrove stick cultivation into the Hawkesbury River the ground referred to was bare of oysters; when, however, large quantities of bundled sticks were laid out on the flat, a prolific crop of oysters was obtained. In Salamander Bay, the sticks, when first laid out, were far removed from any oyster beds, yet here again the initial crop was extremely heavy, and it has been

prolific during almost every season since, despite the fact that practically all of the spat are transferred to other beds, several miles distant, before they have had an opportunity of spawning in Salamander Bay.

After the attachment of the larva as a spat, shell growth becomes much more rapid. In the early stages concentric lines of growth are clearly visible, and the deeply convex larval shell appears to lie on the flatter spat shell like a hood. The larval shell persists for a considerable time before becoming worn off by erosion.

Anatomical Reorganization after Fixation.

Immediately after the larva attaches itself to become a spat, marked changes begin to occur in its anatomy. These have been well described by Stafford (1913) and will be only briefly referred to here. A general rotation of the soft parts within the shell takes place rapidly. The anterior adductor muscle moves upwards and backwards and quickly disappears; the posterior adductor moves downwards and enlarges. Accompanying these muscle movements, the other organs of the young spat move from left to right round the persisting adductor muscle, and the whole axis of the spat is changed. The hinge no longer lies dorsally in relation to the soft parts, but comes to lie anterior to them; the mouth, for instance, instead of lying near the edge of the shell farthest removed from the hinge, in young spat assumes a position below and behind it. As the spat shell develops, the larval shells persist for a considerable time like a hood on each side of the hinge. The velum and foot, no longer of use, are quickly absorbed. Palps develop as a ventral extension of the tissues lying above the anterior extremity of the oesophagus, and the gills extend from the base of the palps to the postero-dorsal border. In very young spat the gills consist only of right and left hemibranchs, the left of much greater depth than the right.

In spat which have attached to glass the movements of the animal within the shell can be clearly followed. The pulsations of the heart are more rapid than in adult oysters and they increase as the temperature rises. When the shells are closed the heart ceases to beat, and the cilia of the gills cease to vibrate. Within the mantles, right and left, the movement of the blood cells can be clearly seen; they are not enclosed in blood sinuses, but move backwards and forwards in a course which is fairly constant in its direction.

Occurrence of the Parasite Bucephalus *in the Gonad.*

During the course of my examination of the gonads of several thousand individuals of *O. commercialis*, I have found five infected with *Bucephalus*, the cercaria of *Gasterostomum*, a trematode parasite which in the adult stage is found in certain species of fish. Two of these oysters were taken from the Hunter River (1927), one from the Hawkesbury River (1927) and two from Cape Hawke (1932). I had previously, in 1924, found the parasites in the gonad of *O. angasi*, taken from the Pambula River. The gonad in each case contained either no sexual products or a few degenerating eggs.

In Europe two species of *Gasterostomum* have been recorded, *G. gracilescens* and *G. fimbriatum*. According to Dollfus (1922) the former develops cercaria (known as *Bucephalus haimeanus*) in the liver (digestive diverticula) and genital organ of *Ostrea*, *Cardium*, *Tapes*, etc.; the cercariae leave these molluscs and enter various species of fish, such as the haddock, whiting, cod, garfish, etc., where they encyst; if these fish are eaten by an angler-fish the larva emerges from its cyst in the intestine and becomes an adult *Gasterostomum*. *G. fimbriatum* is confined to fresh water; the larvae enter mussels (*Unio*, *Anodonta*) in which

they develop into cercariae, known as *B. polymorphus*; from the mussels they enter the rudd (*Leuciscus erythrophthalmus*) where they encyst, and they reach the adult stage in Perch (*Perca*) and Pike (*Esox*). Dollfus states that the ravages of the worm in the European oyster are inconsiderable.

In America, J. Nelson (1914) states that *Bucephalus* infests about 1 per cent. of oysters (*O. virginica*). It is apparently far less common in the Australian oyster. Just what fish form its intermediate and final hosts in Australia I am unable to say.

Summary.

1. The position of the urino-genital clefts of *Ostrea commercialis* and *O. edulis* is compared.

2. The relationship of the gonad to the digestive and other organs is described.

3. The gonoducts open into the urino-genital cleft on each side slightly anterior to the opening of the ureter; they are in close apposition with the visceral nerves (right and left) and are surrounded by sphincter muscle fibres where they enter the clefts.

4. The development of spermatogonia, spermatocytes and spermatozoa, and of ova, from the germinal epithelium of the germinal ducts is described. As the germ cells proliferate in the ducts, the latter evaginate into the vesicular connective tissue internal to them, to form branching and anastomosing follicles.

5. As the gonad continues to develop, the ducts tend to coalesce with their neighbours. In a mature ovary or testis the vesicular connective tissue below the ducts is almost completely absorbed, and that lying between the ducts and the surface epithelium becomes reduced to a very narrow layer.

6. The head of the sperm of *O. commercialis* is spherical and measures 2μ in diameter; the tail is about 42μ long. The eggs are round, oval, or more commonly pear-shaped; they average about 0·05 to 0·06 mm. in diameter, but may occur up to 0·09 mm.

7. Conspicuous amongst the cells of the ciliated epithelium of the germinal ducts are commonly found large, unciliated cells with granular contents, which stain deeply with eosin and other acid stains. These cells have a secretory function and may at times be seen emptying their granular contents into the lumen of the duct. They are most numerous in spawned oysters. Their function has not been determined.

8. The absorption of eggs and sperms which remain in the gonad after an oyster spawns is described. Their place is taken by condensed vesicular tissue, which then continues to grow by absorbing nourishment from the blood stream till it assumes the large bladder-like structure of normal vesicular tissue characteristic of this region.

9. The spawning of the oyster is described. *O. commercialis* was found to spawn on the ebb tide following a high spring tide (new moon). The temperature of the water was 68° F., and the density 1·020.

10. The rate of development of artificially fertilized eggs was found to decrease as the density of the water was lowered.

11. At a water density of 1·005 a large proportion of the eggs remained unfertilized, and in no case did development occur beyond the morula stage.

12. At a temperature varying from 68° F. to 70° F. and with a density of 1·021, the free-swimming stage was reached in 7 hours.

13. The embryos are completely enveloped in shells in 34 hours at a temperature of 68°–70° F.

14. Oyster embryos, 24 hours after fertilization of the eggs, were timed to swim at the rate of 39 inches per hour.

15. At an average temperature of 78° F. the embryos began to swim as trochophores in 6 hours; at an average temperature of 62° F. the same stage was not reached until 22 hours after fertilization.

16. An attempt was made to compute the length of larval life by measuring the larvae taken in the plankton net daily, but at no period during these investigations was there a dominant size-group.

17. The sex-ratio of 3,000 oysters from the principal oyster-bearing grounds of New South Wales was determined. Females were found always to predominate, varying from 54 per cent. to 88 per cent. The average percentage was: females, 73 per cent.; males, 27 per cent. In other words, there were 2·7 times as many females as males.

18. A sex-change is indicated in this species by the fact that practically all, if not all, young oysters spawn for the first time as males. No opportunity was afforded, however, of following the subsequent stability, or instability, of the sex.

19. A total of nine oysters was found (of various ages from 1 to 3 years) which contained both ova and sperms in the gonad. The histology of five of these is described.

20. The determination of sex in this oyster does not appear to be governed by the amount of food available, as has been suggested for other oviparous oysters.

21. Progressive measurements of the larvae are given, and the proportions of the larvae at various stages of growth are compared with those of other species whose dimensions have been recorded. Up to 300μ, the larvae of this species are longer than deep; at about 300μ, the length and depth are equal; from 300μ till they set as spat, their depth is greater than their length.

22. When fully grown the larva measures about 333μ (1/76 of an inch).

23. Young larvae are found in abundance at the surface of the water; older larvae tend to remain near the bottom.

24. Oyster larvae, along with other organisms in the plankton, tend to collect in eddies, and the best spat-catching grounds are those over which the water is caused to eddy by the contour of the land and the tidal flow.

25. More spat attach to the under surfaces than to the upper surfaces of objects in the water, and in water of high salinity than in water of low salinity.

26. The anatomy and movements of the larva are described.

27. The occurrence of the parasite *Bucephalus*, the cercaria of *Gasterostomum*, a trematode parasite which in the adult stage is found in certain species of fish, is recorded from the gonad of five individuals of this species, and of one from *O. angasi*.

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EXPLANATION OF PLATES X-XXVII.

Plate x.

1.—Oyster (*O. commercialis*) with spawn oozing from the gonoduct, situated beneath the adductor muscle.

2.—Oyster with gonad fully developed. The branching ducts show prominently just beneath the surface. Prominent ducts are usually indicative of femaleness, but this oyster was a male.

3.—Oyster with fully developed gonad in which the ducts are not distinguishable at the surface.

Plate xi.

4.—An oyster after a partial spawning. The dark area of the gonad has been drained of spawn.

5.—An oyster after a heavy spawning. The bulk of the gonad has been drained of spawn.

Plate xii.

6.—Sections of an oyster with well developed gonad, showing its relationship to the other organs. The gonad is seen as a whitish mass enclosing the dark digestive diverticula which themselves surround for the most part the intestinal canal.

7.—Photomicrograph of a section of a young oyster, near the anterior extremity of the oesophagus, in which the gonad, in the form of a series of ducts, is beginning to develop. The ducts of the gonad surround the digestive diverticula above and on the sides, but they do not penetrate the vesicular connective tissue between the oesophagus and the digestive diverticula. Bouin: Ehrlich's haematoxylin and eosin. × 40. *d.d.*, digestive diverticula; *d.g.*, ducts of the gonad lined externally with ciliated epithelium and internally with developing germ cells; *e.*, surface epithelium; *m.c.*, mantle cavity; *oe.*, oesophagus; *v.t.*, vesicular connective tissue.

Plate xiii.

8.—The opening of the urino-genital cleft on the right side. Bouin: Ehrlich's haematoxylin and eosin. × 80.

9.—The opening of the urino-genital cleft on the left side. × 80. *b.n.*, branchial nerve; *b.s.*, blood space; *c.*, canal joining mid-gut and style sac; *d.g.*, ducts of the gonad; *h.r.g.*, hemibranch of right gill; *i.*, returning loop of intestine; *k.f.*, kidney follicles; *l.v.n.*, left visceral nerve; *r.v.n.*, right visceral nerve; *m.g.*, mid-gut; *r.m.*, right mantle; *s.b.c.*, supra-branchial chamber; *s.s.*, style sac; *u.c.*, urinary chamber; *u.g.c.*, urino-genital cleft; *v.t.*, vesicular connective tissue of oral process.

Plate xiv.

10.—Section through a genital duct of a developing testis. *c.e.*, ciliated epithelium of duct; *s.c.*, secretory cells in ciliated epithelium; *s.*, spermatozoa; *sp.*^{1}, primary spermatocytes; *sp.*^{2}, secondary spermatocytes; *spg.*, spermatogonia; *v.t.*, normal vesicular connective tissue; *v.t.*^{3}, band of condensed vesicular connective tissue immediately external to the genital duct.

11.—Section through a genital duct of a developing testis. Bles: Ehrlich's haematoxylin and eosin. × 133. *c.e.*, ciliated epithelium of duct; *s.*, spermatozoa; *s.c.*, secretory (eosinophilous) cells in ciliated epithelium; *sp.*, spermatocytes; *spg.*, spermatogonia; *v.t.*, vesicular connective tissue.

Plate xv.

12.—Section through follicles of a developing testis. Bles: Ehrlich's haematoxylin and eosin. × 200. *s.*, spermatozoa; *sp.*, spermatocytes on border of follicle; *v.t.*, vesicular connective tissue.

13.—Section through a mature testis in which the genital ducts have coalesced and the vesicular connective tissue lying between them and the surface epithelium has been reduced to a very thin layer. The testis now consists of an enormous number of closely packed spermatozoa, the tails of which lie in well-defined lanes. Bouin: Ehrlich's haematoxylin and eosin. × 90.

Plate xvi.

14.—Section of a young ovary cut vertical to the surface. Bles: Ehrlich's haematoxylin and eosin. × 64. *d*, genital duct lined by ciliated epithelium externally and germinal epithelium, from which eggs are developing, internally; *d.*^{1}, genital duct from which a follicle is developing by sinking into the vesicular connective tissue; eggs are continuing to develop from the germinal epithelium lining the follicle; *e.*, surface epithelium; *f.*, follicles lined by developing eggs; *v.t.*, vesicular connective tissue.

15.—Section through a young ovary showing a genital duct from which three follicles are beginning to sink into the vesicular tissue. Bles: Ehrlich's haematoxylin and eosin. × 120. *c.e.*, ciliated epithelium of duct; *d.*, cross section of genital duct in which the rapid development of the eggs is causing them to become attenuated and to extend well into the lumen; *f.*, follicles developing from the genital duct and sinking into the vesicular tissue; *f.*^{1}, a branch of a follicle in cross section; *s.c.*, secretory cells in the ciliated epithelium; *v.t.*, vesicular connective tissue.

Plate xvii.

16.—Ovary in an advanced stage of development, showing a duct in cross section. Bles: Ehrlich's haematoxylin and eosin. × 95. *c.e.*, ciliated epithelium of duct; *e.*, surface epithelium; *f.w.*, wall of follicle; *o.*, eggs developing from the germinal epithelium of the duct; continued proliferation has caused many of the eggs to become greatly attenuated; a few have become detached and lie free in the lumen of the duct; *o.*^{1}, eggs attached to wall of follicle.

17.—Section of a mature ovary, showing eggs attached to the walls of the follicles. Bles: Ehrlich's haematoxylin and eosin. × 250. *f.w.*, walls of follicles; *n.*, nucleus; *n.*^{1}, nucleolus; *o.*, eggs.

Plate xviii.

18.—Live unstained eggs of *O. commercialis*. The lighter circular area in each is the nucleus, and in some, the nucleolus, which appears as a fine, clear spot in the nucleus, can be distinguished. × 150.

19.—Section through genital ducts and follicles of a winter male. The ciliated epithelium lining the largest duct externally has been almost completely replaced by greatly distended secretory cells which project well into the lumen of the duct. Bles: Ehrlich's haematoxylin and eosin. × 200. *s.c.*, greatly distended secretory cells; *v.t.*, normal vesicular connective tissue; $v.t.^1$, condensed vesicular tissue filling the duct and the follicle which has developed from it; $v.t.^2$, follicle filled with condensed vesicular tissue.

Plate xix.

20.—Winter male after complete absorption of unspawned spermatozoa. The spermatozoa in the ducts and follicles have been completely replaced by condensed vesicular tissue. Carnoy: Ehrlich's haematoxylin and eosin. × 60. *b.s.*, blood space; *c.e.*, ciliated epithelium lining the ducts; *s.c.*, secretory cells in the ciliated epithelium; *v.t.*, normal vesicular connective tissue; $v.t.^1$, condensed vesicular tissue filling the lumen of the ducts; $v.t.^2$, follicles containing condensed vesicular tissue; $v.t.^3$, condensed vesicular tissue external to ducts.

21.—Winter female in which the unspawned eggs are being absorbed. Bouin: Ehrlich's haematoxylin and erythrosin. × 44. *c.e.*, ciliated epithelium of duct; $o.^1$, degenerating eggs in follicles surrounded by condensed vesicular tissue; *s.c.*, secretory cells in ciliated epithelium; *v.t.*, normal vesicular tissue; $v.t.^2$, portion of a follicle entirely filled with condensed vesicular tissue which has replaced the absorbed eggs.

Plate xx.

22.—Section of a follicle of a winter female showing unspawned eggs in various stages of degeneration. Bouin: Ehrlich's haematoxylin and eosin. × 335. *o.*, normal egg, the absorption of which has not yet begun; $o.^1$, eggs partially absorbed; *v.t.*, normal vesicular tissue; $v.t.^2$, condensed vesicular tissue.

23.—Winter female after complete absorption of the eggs. The eggs in the ducts and follicles have been completely replaced by condensed vesicular tissue. Zenker-formol: Iron haematoxylin and eosin. × 70. *e.*, surface epithelium; *s.c.*, secretory cells which have largely replaced the ciliated epithelium of the ducts; *v.t.*, normal vesicular tissue; $v.t.^2$, condensed vesicular tissue; $v.t.^3$, area of condensed vesicular tissue frequently found scattered throughout the region lying between the genital ducts and the surface epithelium in oysters of various stages of development.

Plate xxi.

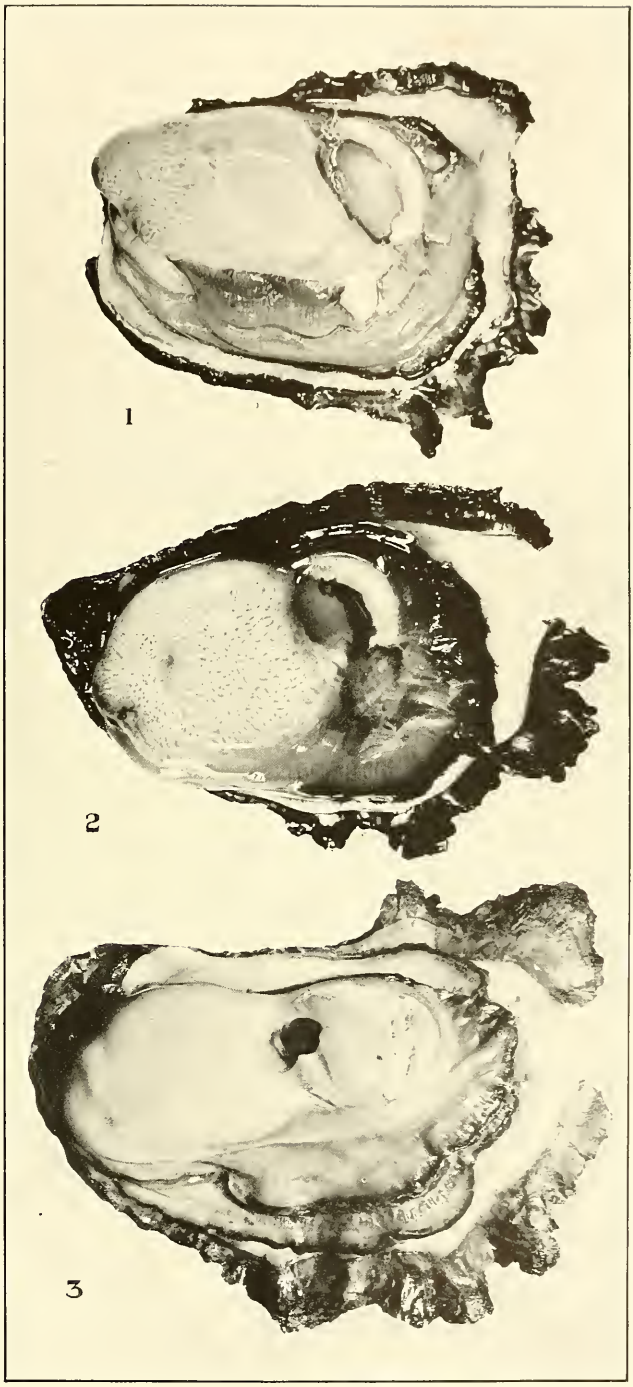
24.—Winter female after complete absorption of the eggs. Zenker-formol: Iron haematoxylin and acid fuchsin. × 65. *b.s.*, blood space; *c.e.*, ciliated epithelium of duct; *e.*, surface epithelium; *s.c.*, secretory cells in ciliated epithelium; *v.t.*, normal vesicular tissue; $v.t.^1$, condensed vesicular tissue filling the lumen of the duct; $v.t.^2$, follicles containing condensed vesicular tissue.

25.—Genital duct and portion of a follicle of a winter female after complete absorption of the eggs. Zenker-formol: Iron haematoxylin and acid fuchsin. × 190. *c.e.*, ciliated epithelium of duct; *v.t.*, normal vesicular tissue; $v.t.^1$, condensed vesicular tissue completely filling the lumen of the duct; $v.t.^2$, condensed vesicular tissue of follicle.

Plate xxii.

26.—Section of the gonad of a hermaphrodite individual of *O. commercialis*, containing eggs and sperms in approximately equal amounts. Spermatozoa (showing as dark masses) are filling the lumen of the ducts, and the germinal epithelium is engaged in egg proliferation. The follicles are filled with spermatozoa and the epithelium lining their walls is for the most part giving rise to ova. Bles: Iron haematoxylin and eosin. × 60. *c.e.*, ciliated epithelium of genital ducts; *d.d.*, digestive diverticula; *e.*, surface epithelium; *o.*, eggs; *s.*, spermatozoa; *v.t.*, normal vesicular connective tissue; $v.t.^3$, layer of condensed vesicular tissue external to genital ducts frequently characteristic of this region.

27.—Portion of a section shown in Fig. 26, more highly magnified. × 445. *o.*, eggs; $o.^1$, developing eggs; *n.*, nucleus; $n.^1$, nucleolus; *s.*, spermatozoa; *sp.*, spermatocytes.



1. Oyster (*O. commercialis*) with spawn oozing from the gonoduct.
2. Oyster with gonad fully developed. The branching and anastomosing ducts show prominently just beneath the surface.
3. Oyster with fully developed gonad in which the ducts are not distinguishable at the surface.